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CADMIUM IN THE ENVIRONMENT - III

A TOXICOLOGICAL AND EPIDEMIOLOGICAL APPRAISAL

KAROLINSKA INSTITUTET

PREPARED FOR
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A TOXICOLOGICAL AND EPIDEMIOLOGICAL APPRAISAL

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16. ABSTRACT

This report is a review of the recent biomedical literature on cadmium. The major part of the material covered is from 1973-74, but some earlier work not contained in volumes I and II is included. The recent findings are discussed in relation to previous work where this is appropriate.

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CHAPTER 1. INTRODUCTION

This volume constitutes the third report on cadmium written under contract No. 68-02-1210 between the U.S. Environmental Protection Agency and the Department of Environmental Hygiene of the Karolinska Institute, Sweden. Previous data are available in the two earlier reports and in a monograph published by the Chemical Rubber Company Press and entitled Cadmium in the Environment, 2nd edition, 1974 (in this report abbreviated as CITE, 2nd ed.).

Since we received the first contract to compile and evaluate data on cadmium, an upsurge of interest, research and publication about this metal has been witnessed. Several reviews have appeared, those of Flick, Kraybill, and Dimitroff, 1971; Riihimäki, 1972; Fulkerson et al., 1973; and Fleischer et al., 1974, to name just a few. In addition, the Oak Ridge National Laboratories have put out an extensive annotated bibliography entitled Cadmium in the Environment and edited by Emily Copenhaver et al. A health criteria document on cadmium is currently under preparation at WHO.

Like the previous reviews, the present work focuses upon information essential to the understanding of the toxic action of cadmium and the relationship between exposure and effects on human beings and animals.

Through repeated personal contact with several Japanese researchers, it has been possible to obtain and evaluate much data from Japan which would not have been accessible otherwise. Several articles in Japanese were translated through resources provided by the Project Officer. Non-translated articles in the Japanese language could be taken into account as well since Dr. Kjellström speaks and reads Japanese. We express our gratitude for invaluable assistance from different Japanese researchers and agencies.

CHAPTER 2. PROBLEMS OF ANALYSIS

In CITE, 2nd ed., it was concluded that several methods may be used for the determination of cadmium in biological material, such as food, urine, organs, blood, etc. The most commonly used method was reported to be atomic absorption spectrophotometry, but interferences from especially NaCl may give erroneously high results. Extraction techniques or background correction techniques could limit the problems of interferences.

The great lack of strict methodological studies and of comparisons of analyses of the same material between different laboratories was pointed out. In the chapters about occurrence and metabolism of cadmium in CITE, 2nd ed., the difficulties in comparing data from different countries, when the accuracy of the methods is uncertain, were discussed.

Because of the different composition of various biological materials no standardized methodology suitable for all materials has been developed. Especially at the low concentrations of cadmium, occurring in for instance urine and blood of non-exposed persons, considerable problems still remain as regards finding suitable methods for cadmium analysis.

Shults, Lyon and Carter, 1973, compiled the available information about different methods for cadmium analysis, making a rough calculation of the sensitivity and accuracy of each method. The performance of the methods is to a large extent dependent on the chemical preparation of the samples before final analysis and therefore their figures should be considered approximate and dependent on the type of material analyzed.

In CITE, 2nd ed., the problems of analysis were discussed method by method, but in this report we have chosen to present the new data in relation to the type of material analyzed instead.

2.1 ANALYSIS OF FOODSTUFFS

Childs and Gaffke, 1974, investigated the interferences of different elements with measurement of lead and cadmium in fish muscle. Interfering solutions of 14 different elements were prepared at the concentrations regularly found in fish muscle. Such solutions were added to standard solutions of cadmium and these mixtures were analyzed with flame atomic absorption either directly or after extraction with MIBK. Interferences were regularly less than 10% at a cadmium concentration of 0.1 μg/g in water solution, but some elements (Al, P, Na) decreased cadmium measurements with more than 10% and some (Ba, Zn, Cl) increased the measurement with more than 10% after MIBK extraction.

Gajan et al., 1973, described a standard method for determination of cadmium in food, which was adopted by the Association of Official Analytical Chemists (AOAC). The method, involving wet ashing and extraction in dithizone/chloroform followed by atomic absorption, had been tested in a collaborative study. Detailed instructions for this method were distributed to 10 different laboratories, each of which first practiced on samples with known cadmium concentrations. Unknown samples of various foodstuffs spiked with 0.05 μg/g or more were subsequently distributed and analyzed by each laboratory. In this way the recovery of the different laboratories could be calculated, turning out to be between 83 and 116% for each material and spiking level. The average results on duplicate unspiked samples of lettuce ranged from 0.02-0.04 μg Cd/g, for sugar from 0.00-0.05 μg/g and for wheat from 0.01-0.1 μg/g.

A chelating ion exchange resin has been used by Baetz and Kenner, 1974, for the separation of cadmium from food samples digested with HNO_3, H_2SO_4 and H_2O_2. Cadmium is analyzed with atomic absorption spectrophotometry without background correction. This method was demonstrated to be comparable with the method by Gajan et al., 1973, mentioned above, both methods giving about 90-100% recovery after spiking with 0.4 or 0.3 μg Cd/g. For original foods on the other hand the detection limit was 0.01 μg Cd/g. In several materials, as for instance sugar, beans and rice cadmium was not detectable. At this low level the agreement was not good, and it is doubtful whether the conclusion, that the ion exchange resin method allows determination down to 10 ng Cd/g, is valid.

A pulse polarographic technique for analysis of cadmium in dried milk has been developed by Cornell and Pallansch, 1973. 10 g samples of milk powder were dry ashed at 500°C overnight and the ash was dissolved in diluted nitric acid. The solution was analyzed in a polarographic analyzer using a hanging mercury drop electrode. Cornell and Pallansch tested different ashing temperatures and nitration of ash during ashing and found that at 470°C and lower the recovery was 5-10% lower than at 500°C. This could be improved by nitration of the ash during the ashing procedure. It was believed that carbon particles caused a decrease in the recovery. When the temperature during ashing was raised to 600°C the recovery also decreased, probably due to volatilization of cadmium.

When the dry milk samples were spiked with 2 ng Cd/g and 10 ng Cd/g, recovery at the lower level was about 86% and at the higher level about 95%. The natural concentration of cadmium in dried skim milk was about 2 ng/g. In dried cow milk the authors found 0.7-2.9 ng Cd/g in 18 individual determinations, the relative standard deviation being 28%. The useful lower analytical limit for routine analysis of milk powder is claimed to be 0.5 ng Cd/g, corresponding to about 0.05 ng Cd/g wet weight in fluid milk. The authors noted that the procedure developed for milk should be applied to other material with caution.

Lee, Kim and Park, 1973, described a method for simultaneous neutron activation analysis of mercury, bromide, arsenic and cadmium in biological samples. The cadmium method is similar to earlier reported methods for neutron activation analysis. In a standard kale powder with an earlier reported average cadmium concentration of 0.74 μg/g, the authors reported 0.50±0.05 μg Cd/g, but left this difference without discussion. On 3 rice samples from Korea they reported 0.02±0.002, 0.075±0.004 and 0.10±0.007 μg Cd/g. These results are in the same range as reported cadmium concentrations in rice from non-polluted areas in Japan (CITE, 2nd ed.).

Krinitz and Franco, 1973, suggested a standard method for determination of cadmium and lead extracted from glazed ceramic surfaces, and compared results from 13 different laboratories when they used this method. The method is adapted from the German standard procedure, and involves leaching of the metals into 4% acetic acid at 22°C for 24 hours. The leaching solutions are analyzed with flame atomic absorption spectrophotometry. In leaching solutions with an average of 1.01 μg Cd/ml the relative standard deviation among the 13 laboratories was 3.9%. Most of the paper deals with leaching of lead, and the evaluation of the performance of the method on cadmium containing samples is not extensive. This method was adopted by the AOAC.

Kjellström et al., 1974a, compared the results of cadmium analysis in wheat from 7 different laboratories, each laboratory using its own method. The samples, all unspiked, ranged from about 10 to about 115 ng Cd/g. The analysis was performed on a completely blind basis. There was a good agreement between neutron activation analysis, sparked source mass spectrometry, isotope dilution and atomic absorption spectrophotometry after high temperature dry ashing or wet ashing. On the other hand atomic absorption without background correction and emission spectroscopy gave considerably higher values (Table 2:1).

2.2 ANALYSIS OF URINE

Recently the heated graphite atomizer (HGA) and the carbon rod atomizer (CRA) have been introduced as tools to increase sensitivity in atomic absorption spectrophotometry. Kubasik and Volosin, 1973, have developed a method for the simultaneous extraction of lead, cadmium and thallium from urine with a combination of sodium diethyldithiocarbamate and methylisobutylketone (DDTC/MIBK). 1.6 microliters of extracted metal solution were introduced into the carbon rod atomizer. With an addition of 5 μg cadmium per liter urine, the recovery was 90-104% in 10 samples. The authors showed that the standard curves for unextracted aqueous standards and extracted urine standards differed in such a way that the urine standards of cadmium were about 30% lower. Some constituents in the urine matrix affected the sensitivity of the standard curve, whereupon the authors suggested that standard additions for preparation of the standard curve are needed for accurate analyses. A hydrogen lamp was used for background correction and after extraction the authors reported that the non-atomic absorption accounted for about 0.5 μg cadmium per liter. When urine was injected directly into the carbon rod atomizer the results were unsatisfactory because of the great non-atomic absorption. The coefficient of variation in 20 determinations on a urine pool was 4% (average cadmium concentration 6.15 μg per liter). In 13 samples of unspiked normal urines, the average was 1.7 μg cadmium per liter (range 0-4.1 μg cadmium per liter). Kubasik and Volosin did not discuss the possibility of a larger coefficient of variation at this low cadmium level.

Kubasik and Volosin, 1973, also studied the effect of addition of various elements to the urine before extraction, showing that a fivefold increase in chloride concentration of urine gave 122% recovery of cadmium and a fivefold increase in phosphate concentration gave 84% recovery of cadmium as compared to a 96-103% recovery of cadmium when 7 other elements were added.

Interlaboratory comparisons of cadmium analyses in urine have recently been performed. Hoschek and Schittke, 1973, reported on a study in which a number of German research institutes received similar subsamples of 10 urine samples for analysis of lead and cadmium. The samples were prepared from a mixture of urine to which cadmium from 10 μg per liter to 1,000 μg per liter had been added. Only the data from one institute regarding cadmium, for which the dithizone method was utilized, are presented in the report. It is seen that the agreement between expected cadmium concentrations and found cadmium concentration was very good in the range of 10 μg per liter to 1,000 μg per liter.

Kjellström et al., 1974a, compared cadmium analyses performed in 3 Japanese and 1 Swedish laboratory on urine samples (range 1-28 μg/l) using neutron activation analyses and atomic absorption spectrophotometry with different modifications of the preparatory steps. As seen in Figure 2:1 the agreement between neutron activation analyses and the different atomic absorption methods was good. One of the APDC/MIBK extraction methods used at the Swedish laboratory gave somewhat lower values than the other methods. The low level samples were a mixture of normal Swedish urines and the high level samples were either spiked with cadmium standard or mixtures of urine from cadmium workers.

Shiroishi et al., to be published, made another interlaboratory comparison of cadmium analyses. The participants were one of the Japanese laboratories which contributed to the study by Kjellström et al., 1974a (using AAS after DDTC/MIBK extraction) and the same Swedish laboratory (using AAS after APDC/MIBK extraction). The analyses were performed on a blind basis. There was no difference in the average results, the correlation coefficient between the results from the laboratories being 0.99.

Harada, Taniguchi and Sato, 1974, studied the cadmium concentration in urine from 117 persons living in a cadmium polluted area. 50 ml of urine was wet ashed with nitric acid and perchloric acid and afterwards extracted with APDC/MIBK. Cadmium concentration was analyzed with atomic absorption spectrophotometry with and without deuterium background correction. The average uncorrected cadmium concentration was 13.53 μg per liter and the average corrected concentration 6.52 μg per liter. It is surprising that such a difference in these values would be received after APDC/MIBK extraction. Harada, Taniguchi and Sato, 1974, also analyzed 15 of the urine samples with atomic absorption after dithizone/chloroform extraction. On an average the dithizone/chloroform technique gave about 10% lower values than APDC/MIBK without correction.

2.3 ANALYSIS OF ORGANS

In adult human beings or in exposed animals the cadmium concentrations in organs like lung, liver and kidney are usually so high (Chapter 4) that several existing methods may be considered reliable. Two new approaches in this field will be reported here. Kaplan, Blackstone and Richdale, 1973, have modified the chemical preparation of lung samples before atomic absorption. They digest dried lung samples in tetramethylammoniumhydroxide in toluene at 60°C for 24 hours after which they analyze the toluene solution directly with flame atomic absorption spectrophotometry. In the description at hand, 0.25-gram samples of rat lung tissue were analyzed, giving a coefficient of variation of between 2 and 25% at cadmium levels between 0.3 and 6 μg/g (3 determinations). The method was not compared with any other method, hindering the evaluation of the advantages of this type of sample preparation.

Another new approach in cadmium analysis is neutron activation analysis in vivo where a neutron flux from a lithium target is directed to a part of a living person (Biggin et al., 1974). The neutron flux is given in pulses of 15 microseconds. During half an hour the person receives a dose of 1 rem. The neutrons are captured by the stable isotope ^{113}Cd (12.3% abundance), followed by emission of a cascade of gamma rays where the strongest energy is at 559 keV.

In a study on phantoms Biggin et al. found that the detection limit of the method would be around 2 μg/g for the liver. However, the authors reported that in a study on a cadaver, they could only ascertain the presence of cadmium in the region of the liver. No specific concentration could be calculated. It is thus still uncertain as to whether this in vivo neutron activation analysis can be developed into a feasible method for cadmium analyses.

2.4 ANALYSIS OF BLOOD

In CITE, 2nd ed., it was concluded that analysis of cadmium content in blood poses considerable problems and that the true average blood concentration among non-exposed persons is not known but can be assumed to be around 1 ng/g or less. For exposed persons spectrographic methods have been used and the practical detection limit was around 4 ng/g. As with urine (section 2.2) the matrix effects when analyzing blood may be considerable.

A method which has been given considerable interest during recent years is the "Delves cup" technique applied to atomic absorption spectrophotometry. The Delves cup technique involves introducing a micro-crucible made of nickel into the flame of the atomic absorption spectrophotometer and in this micro-crucible, 10 μl aliquots of blood are introduced and atomization of blood cadmium achieved. The method was primarily developed for blood lead analysis (Delves, 1970). In order to avoid the matrix effects when analyzing cadmium in blood with the Delves cup technique, Lieberman, 1973, extracted cadmium from hemolyzed, heparinized whole blood with APDC/MIBK. This direct extraction of cadmium from blood has also been used by Westerlund-Helmerson, 1970, in combination with regular flame atomic absorption and then the detection limit was claimed to be 2 ng Cd/g blood. Lieberman, 1973, used 4 ml samples of whole blood and by adding cadmium chloride equivalent to concentrations varying between 5-100 ng Cd/ml blood, he found the apparent sensitivity of the technique to be approximately 1 ng/ml. On the other hand in triplicate analysis of the sample with 5 ng Cd/ml added the range in absorbance was 0.022-0.038 indicating that at low cadmium levels the reproducibility of the method is rather poor. With added cadmium concentrations equivalent to 50-100 ng Cd/ml the author found the recovery to be close to 100% for both urine, plasma and whole blood. As the cadmium additions in the study were equivalent to cadmium concentrations 5-100 times higher than the expected values in non-exposed persons, it is hard to draw conclusions about the validity and precision of this method at lower levels.

Cernik, 1973, put 20 μl whole blood samples into nickel micro-crucibles (Delves cup), dried the samples at 100°C for one minute and then oxidized the dried blood samples by heating them on a hot-plate at 425°C for 5-6 minutes. When the micro-crucibles were subsequently put into the flame, a single peak could be received on the recorder corresponding to the cadmium atomization. Background absorption, checked at 220 nm with a lead lamp, never accounted for more than 3%. Internal standards were used. By adding cadmium to blood corresponding to 20 ng/g it could be shown that the losses of cadmium in the drying and oxidation steps varied between 6 and 10%. By adding cadmium corresponding to 25 ng Cd/ml at repeated analysis the standard deviation was about 2 ng Cd/ml and the absolute sensitivity of the method 23 picogram cadmium (corresponding to 1.15 ng Cd/g blood). In this case as well, the method was not tested on the low cadmium levels actually found in "normal" samples.

A problem with the Delves cup is that its repeated insertion and removal from the holders may give rise to small distortions in the shape of the micro-crucibles which seriously affect the precision of the method (Joselow and Singh, 1973). A good precision even after 200 analyses can be achieved by not removing the cups from the holder between burn offs.

Another problem with the technique is the precision needed as regards the distance from cup to flame in the initial combustion phase and position of the cup in relation to the absorption tube (Ulander and Axelson, 1974). These authors developed a mechanical device with which the position of the micro-crucible could be reproduced with precision (Ulander, personal communication). Whole blood was placed into the micro-crucibles without extraction and in 25 repeated analyses of blood samples with addition of 1, 3, 10 and 20 ng Cd/g as well as without addition it could be shown that the mean blood concentration without addition was 1.4 ng/g with a coefficient of variation of 16%. The range was 1.1-1.8 ng Cd/g (n = 25). With increasing cadmium additions the coefficient of variation decreased and with an addition equivalent to 20 ng Cd/g the average cadmium concentration found was 21.4 ng Cd/g with a coefficient of variation of 6.3% (n = 25). The data indicate that the Delves cup technique performed in the way described by Ulander and Axelson, 1974, can also give reproducible results at the low cadmium concentrations normally found in non-exposed populations. The problems are nonetheless considerable and Delves, 1974, himself reported that it was not possible for him to separate the cadmium absorption signals from those of the combustion products of the sample when using the Delves cup technique, because of the relatively low vapor pressure of cadmium and its salts. Further research on the methodology of the Delves cup technique is needed in order to develop a reproducible and dependable standard procedure.

Stoeppler et al., 1974, used the flameless atomic absorption technique for analyzing cadmium in water-hemolyzed whole blood without extraction. The average in 37 samples was 9.5 ng/g (range: 4-19 ng Cd/g). The authors themselves believed this value to be rather high but could not find an explanation. Deuterium background correction was applied. The problems involved in blood cadmium analysis are underlined by results of an interlaboratory comparative study on analysis of the same samples (Berlin et al., 1974). Three blood samples were sent to 14, 17 and 17 different laboratories respectively. The range in the average results reported from each laboratory was 1-92, 0.3-73, and 0-110 ng/g for the three blood samples.

One aspect of blood cadmium analysis that is sometimes ignored is the contamination of metals from syringes and blood containers during sampling. Taylor and Marks, 1973, filled different containers with distilled water and left them for 48 hours at room temperature. In some of the containers up to 400 ng zinc per gram was found in the distilled water after the experiment and up to 100 ng magnesium/g. Syringes and pistons of syringes were tested by putting them in 10 ml of distilled water for 30 minutes at 75°C. Metal concentrations found in the water after the experiment were up to 2,000 ng zinc/g, up to 430 ng copper/g, and up to 560 ng magnesium/g. As it can be assumed that together with zinc contamination, cadmium contamination may also occur to a certain extent, the data indicate the necessity of checking equipment for metal contamination.

2.5 ANALYSIS OF AIR

Another material which is important to analyze in health effect studies is air, both ambient and workplace. It is not the aim of this report to go into detail concerning all the problems regarding analysis of air related to the length and interval between sampling times, locations of sampling sites, procedure for dust collection and the chemical analysis of cadmium in collected dust. The general aspects of air analysis have been treated in a recent review by Linch, 1974.

Some details worth mentioning are that Natusch, Wallace and Evans, 1974, found a preferential concentration of some heavy metals in small-sized fractions of dust in air. Both regarding cadmium and several other elements the concentration increased when the particle size decreased. The authors offered one explanation as being that the elements volatize in the high-temperature combustion in emitting-industries and then recondense preferentially or are adsorbed preferentially onto the large available surface area per unit mass provided by the small particles. The authors conclude that the highest concentrations of toxic elements are emitted in the smallest, lung-depositing particles and that particle collection devices should be designed for collecting the smallest particles. They also concluded that the estimates of toxic element emissions based on analyses of samples from existing particle precipitators would grossly underestimate the actual emissions.

MacLeod and Lee, 1973, investigated a method for trace metal determination of spot-tape samples (AISI-method) of ambient air. Anodic stripping voltammetry after low-temperature ashing of cut-outs of tape from the automatic tape sampler was employed. The problem with this methodology was that the blanks (spot sampler tapes without dust) contained 44-116 ng Cd. Because of the large variation of cadmium amount in the blanks and the size itself of this amount, the practical lower level for cadmium analysis would be around 500-1,000 ng/sample. As these samples contained only about 60% of the dust collected, the total necessary cadmium amount in collected dust would be around 1.5 μg. In order to analyze cadmium concentrations down to 1 ng/m^3 more than 1,000 m^3 of air has to be sampled in one filter. Colovos, Wilson and Moyers, 1973, also applied anodic stripping voltammetry analysis of cadmium in dust collected on millipore filters. Low temperature ashing was utilized. The millipore filter contains less cadmium in the blanks; the authors claimed that the practical sensitivity of the method was 10 ng Cd/filter which means that at least 10 m^3 of air must be sampled in order to detect 1 ng Cd/m^3.

Kneip et al., 1973, suggested a dithizone/chloroform extraction method combined with light spectrophotometry for analysis of air samples. The authors stated that in order to analyze 10 ng Cd/m^3 an air-sample volume of 200 m^3 would be appropriate. In cases in which very limited air volumes can be sampled the method by Brodie and Matousek, 1974, may be the most suitable. Air dust is collected on millipore filters, the filters having been washed in hydrochloric acid and distilled water prior to use in order to reduce the blank. A cut-out of the filter is inserted directly into a carbon-rod atomizer and treated therein with 2 μl of phosphoric acid (1,000 μg/g). Cadmium phosphate has a relatively high boiling point and the atomization comes somewhat later than for other cadmium compounds, hence enabling a better separation of non-atomic absorption and cadmium absorption in the recorder. The atomic absorption spectrophotometer used by Brodie and Matousek was only single beam and therefore deuterium background correction could not be applied. Very limited data are presented in the paper, but the authors claim that the absolute sensitivity of the method would be 1.5 picogram cadmium, which corresponds to an air concentration of 8 ng Cd/m^3 in an air volume of 0.2 liters! Further research into this analytical method would be of great interest.

2.6 ANALYSIS OF WATER

Rojahn, 1972, analyzed estuarine water from the Oslofjord by anodic stripping alternating current voltammetry. Five similar samples gave an average cadmium concentration of 0.19 μg Cd/liter (variation coefficient = 21%) and the average of a duplicate analysis of a similar sample using atomic absorption spectrophotometry was 0.12 μg Cd/liter. Prior to anodic stripping voltammetry, the samples had been filtered through a 0.45 μm membrane filter. Atomic absorption was performed after extraction. The data show the relatively large differences that may be obtained even in such relatively uncomplicated samples as water.

Olsen and Sommerfeld, 1973, suggested that the following procedures could be suitable for storage of extracted water samples. 500 ml samples of water were extracted with DDTC/MIBK technique and subsequently the MIBK layer (approximately 40 ml) was evaporated to dryness under partial vacuum at 50°C and to this 35 ml of 1:1 acetone: 0.1 N HCl was added. Such acetone HCl metal-containing solutions were analyzed immediately with flame atomic absorption spectrophotometry, and again after two weeks, in order to be compared with freshly extracted samples. The difference between the freshly extracted samples and the two-week old samples in acetone-HCl solution was negligible at a concentration of 20 ng Cd/g, which had been added. The original cadmium concentration in lake water could not be detected.

2.7 CONCLUSIONS

The recent awareness of the importance of accurate determination of cadmium in biological fluids and food has resulted in a large number of publications.

Some methods presented in this chapter seem to be able to determine levels of 0.01-0.05 in food relatively accurately. Accurate values for cadmium concentrations in blood and urine from exposed persons should be possible to obtain by several of the methods presented. It is not quite clear, however, how accurate these methods are for determination of "normal" levels in blood and urine. Collaborative studies using different methods are needed.

TABLE 2:1

COMPARISON OF ANALYSIS OF CADMIUM CONCENTRATION IN GRAINS (2ND ROUND). DUPLICATE ANALYSIS WHEN RANGES ARE GIVEN, UNIT = ng Cd/g WET WEIGHT. (From Kjellstrom et al., 1974a).

Laboratory		Sample No. (harvest year)				Estimated variation coefficient (V)
		1 (1971) wheat	3 (1945) wheat	4 (1971) oats	5 (1941) wheat	
Neutron	r	41 - 48	23 - 26	12 - 17	22 - 22	14.1 %
activation	a	44.5	24.5	14.5	22.0	
Keio* University	a	120	60	50	100	
Institute of*	r	133 - 144	67 - 100		107 - 111	16.5 %
Public Health	a	139	84	84	109	
Karolinska**	r	20.7 - 40.6	17.1 - 18.3	6.1 - 7.2	14.5 - 21.6	27.6 %
Institute	a	30.7	17.7	6.7	18.1	
ERS, USA †	a	220	53	210	65	
Toyama Inst.***	r	44 - 51	32 - 36	20 - 21	23 - 28	9.8 %
of Hygiene	a	47.5	34.0	20.5	25.5	
Okayama** University		44	33	17	79	
National †† Bureau of Standards	x	41	20	12	35	

r = range a = average x = only one figure significant

* = low temperature dry ashing AAS without background correction.

** = high temperature dry ashing AAS.

*** = wet ashing, extraction AAS.

† emission spectroscopy.

†† spark source mass spectrometry isotope dilutions.

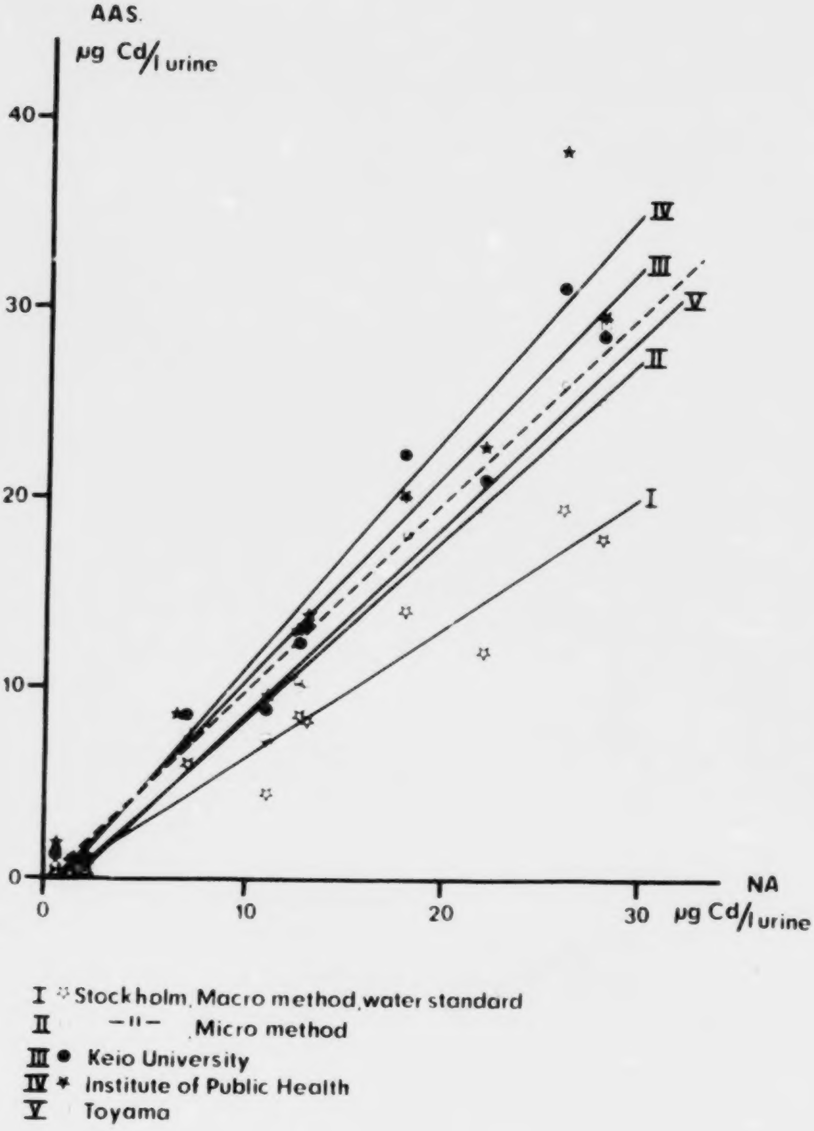

FIGURE 2:1 Comparison of cadmium analysis of urine. Neutron activation analysis used as a "reference method" on the abscissa. The ordinate gives results of AAS from the five laboratories. Linear regression lines depicted. (From Kjellström et al., 1974a).

CHAPTER 3

OCCURRENCE, POSSIBLE ROUTES OF EXPOSURE AND DAILY INTAKE

In CITE, 2nd ed., data were given on the levels of cadmium in different compartments of the environment and in food. It was concluded that exposure via food was the most important route for the general population, but that near cadmium-emitting industries significant amounts of cadmium could be inhaled. Smoking cigarettes could also cause a significant absorption of cadmium.

In the following only new data of special interest will be discussed. Thus there is no section on cadmium in water, since there are no new data which could pose changes or meaningful supplementation of earlier conclusions. Reference is also made to a recent review by Page and Bingham, 1973, which will not be dealt with here, on cadmium residues in the environment.

3.1 OCCURRENCE AND POSSIBLE ROUTES OF EXPOSURE

3.1.1 Cadmium in air - occurrence and sources

In CITE, 2nd ed., it was reported that in rural areas monthly means of cadmium concentrations in air were generally around 1 ng/m^3 or less, whereas in industrial areas up to 50 ng/m^3 had been recorded and near point sources much higher values could be reached.

Data have now been reported by Janssens and Dams, 1974, from Belgium, one of the large zinc and cadmium producers in Europe. On two occasions in 1972, with west and east winds respectively, 24-hour collections were made in 13 residential and industrial areas distributed throughout the country.

The method of analysis was flameless atomic absorption using a graphite furnace after ultrasonic treatment of cellulose filters with a 0.1 M nitric acid. The authors studied different pretreatment methods, interference, and other parameters. With due regard to the difficulties involved, they stated that all the methodological testing suggested a good accuracy.

At the coast 1 ng/m^3 (west wind) and about 30 ng/m^3 (east wind) were recorded. In the industrial area in Liège 150 (westerly) and 441 (easterly) ng/m^3 were recorded and in the Liège residential area 50 (westerly) and 507 (easterly) ng/m^3. In Brussels 10 (westerly) and 60 (easterly) ng/m^3 were found.

These data suggest that the air levels of cadmium in Belgium could have a high average. The short sampling period makes it impossible to estimate monthly or yearly means.

Further evidence of transport of cadmium from zinc smelters has been provided by Little and Martin, 1972, Ruiz and Acero, 1972, and Buchauer, 1973. In the latter study background levels of cadmium were reached first at 15-20 km from the smelter.

Air concentrations of cadmium at different distances from a factory producing copper-cadmium alloys were determined during two 6-month periods by Lind and Piscator (unpublished data). The first sampling period was before a cleaning equipment had been installed and the second after installment. In Fig. 3:1 it is shown that a considerable reduction in air levels was obtained and background levels of cadmium were reached only a couple of km from the emission source.

The interest in increasing the use of coal as an energy source has resulted in a number of reports on cadmium in coal. Toca, Cheever and Berry, 1973, collected particles according to size from the effluent from a coal-fired boiler. It was calculated that 68% of the cadmium was in particles with MMD less than 5 μ. Gluskoter and Lindahl, 1973, determined cadmium in 23 Illinois coals. The range was from 0.3 to 28 μg/g. Sorensen, Kober and Petering, 1974, determined cadmium in coal from Pennsylvania and Utah, all samples being found to contain less than 1 μg/g.

Fly ash from coal-fired power plants in USA was analyzed by Natusch, Wallace and Evans, 1974. Airborne fly ash was separated according to particle size and it was found that cadmium concentrations in the particles increased with decreasing particle size.

An extensive study on metal release from a coal-fired power plant has been reported by Bolton et al., 1973, 1974. Cadmium was followed from its occurrence in coal to deposition in soil and water. This power plant was found to cause relatively small increases in cadmium levels, e.g. in soil cadmium concentrations varied between 0.3 and 1.5 μg/g within 5 miles from the plant. The coal used contained 0.47 μg Cd/g.

Cadmium has been analyzed in oil imported to Sweden (Andersson and Grennfelt, 1973). In all samples tested the concentrations were less than 10 ng/g.

3.1.2 Cadmium in soil and uptake by plants

In CITE, 2nd ed., it was concluded that cadmium levels in uncontaminated or slightly contaminated areas were on an average well below 1 μg/g, whereas values from areas near cadmium-emitting industries were far above 1 μg/g. Important foodstuffs like wheat and rice will take up cadmium from soil. The pH is also a key factor. The presence of high amounts of cadmium in sewage sludge and other fertilizers was also pointed out.

Extensive studies on cadmium in soil and its uptake in plants have been performed in Japan by Takijima and co-workers (Takijima and Katsumi, 1973a, b; Takijima, Katsumi and Takezawa, 1973; Takijima Katsumi and Koizumi, 1973a, b).

Among the findings can be mentioned that analysis of rice field soil downstream from a zinc mine revealed cadmium concentrations ranging from 0.2 to 10.4 μg/g. In rice the concentrations found were from 0.02 to 1.82 μg/g. There was, however, no significant correlation between soil cadmium and cadmium in rice (Takijima and Katsumi, 1973a).

A highly significant negative correlation was found between soil pH and the cadmium content of rice (Takijima, Katsumi and Takezawa, 1973). In pot experiments it was found that poultry manure decreased the uptake of cadmium by an increase in pH, whereas rice straw compost did not have that effect (Takijima, Katsumi and Koizumi, 1973a). In further experiments it was shown that treatment of soil with a combination of calcium silicate and fused magnesium phosphate was most effective for reducing cadmium concentrations in rice, resulting in concentrations about one fifth of the control (Takijima and Katsumi, 1973b). By treating a flooded paddy field with hydrochloric acid (0.1 M) one-fourth of the soluble cadmium could be removed. After drainage and washing the field was treated with calcium silicate and magnesium phosphate. This resulted in a reduction in cadmium concentration in rice from 0.33 µg/g to 0.06 µg/g (Takijima, Katsumi and Koizumi, 1973b).

The mechanisms behind the uptake of cadmium by barley have been studied by Cutler and Rains, 1974. They concluded that the most important mechanism was diffusion and continuous fixation of cadmium to binding sites in the barley roots.

The uptake of cadmium from fertilized soil has been extensively discussed by Williams and David, 1973. They found cadmium concentrations in superphosphate ranging from 27 to 48 µg/g (atomic absorption after extraction with dithizone) and the application of superphosphate during the years had increased the cadmium content of soil about 5-fold. Since analysis of wheat grain revealed low cadmium concentrations (0.012-0.036 µg/g) they concluded that the fertilizing had little effect on cadmium concentrations in grains. That commercial fertilizers may contain large amounts of cadmium has also been shown by Stenström and Vahter, 1974.

Huffman and Hodgson, 1973, collected samples of wheat and various types of grass from about 100 sites in East and Central USA.

4-gram samples were dry-ashed overnight at 450^{o}, then wet-ashed in nitric acid, and finally dissolved in 10 ml 0.1 N HCL. Cadmium and zinc were determined directly in the solution with regular flame atomic absorption, probably without deuterium background correction. The recovery was checked with added standards equivalent to 0.5 µg Cd/g and ranged between 85-125 %. The total average cadmium concentration in 33 wheat samples was 0.20 µg Cd/g dry weight. In 120 samples of grass the average cadmium concentration was 0.17 µg Cd/g dry weight. The zinc/cadmium ratio in wheat was 88 and in grass 137.

These data indicate up to 4 times as high cadmium concentrations in American wheat as in Swedish wheat (Kjellström et al., 1974b). Only a part of this difference could be due to the drying of the samples as usually the ratio between dry weight and wet weight for wheat grains is about 0.9. The lack of background correction in the analysis may be one reason for the variation in recovery reported by Huffman and Hodgson, 1973, and the average cadmium concentration in wheat may hence be somewhat too high. The authors formulated the hypothesis that there should be a co-variation between incidence of hypertensive disease and cadmium concentration in plants but such a co-variation was not shown in the reported data.

Koshino, 1973, studied the yield and metal content in rice and wheat in relation to addition of metals and various types of fertilizer to seed. The method of cadmium analysis in grains was based on low temperature ashing, dissolving the ash in 1 N HCL and directly analyzing this solution with flame atomic absorption without background correction. This method may not be suitable for low level samples (section 2.1) but at the µg/g-level reliable data are probably received.

It was reported by Koshino that superphosphate contained 13.8 and 15.4 µg Cd/g wet weight in two samples. "Cd-free" (<1 µg Cd/g) P_2O_5 was added together with Cd to 10 kg (wet weight) seed in pots. The soil contained in itself 0.4 μg Cd/g (soluble in 0.1 N HCl), and had a pH of 5.9. As seen in Table 3:1 the Cd concentration in wheat grains increased when Cd was added. The yield of grains decreased considerably when more than 30 mg Cd had been added. It was 82 % as compared to the controls at 100 mg Cd addition (1.5 g P_2O_5) and 11 % and 3 % respectively when 250 and 500 mg was added. Liming of the soil decreased Cd uptake and this may be an effect of a higher pH in soil (Table 3:2).

Similar results were received for rice but the effects on yield were more marked for wheat. Simultaneous addition of manganese, iron and low levels of zinc alleviated the effects, giving higher yields; copper on the other hand aggravated the damaging effect.

The possibility of utilizing cedar trees as indicators of metal pollution was tested by Ishizawa et al., 1974. The study was performed in the vicinity of the now defunct Sazagawa mine in the western part of Shimane Prefecture, Japan. There is a history of agricultural damage from pollution by the mine, where copper and arsenic were produced for 600 years, ending in May, 1971. Eleven cedar samples from a control area and 29 cedar samples from the polluted area, along a river coming from the mine and the smeltery, were taken for analysis. At the same places, soil samples were also collected, from which the fraction with a particle size less than 2 μm was analyzed. Cedar leaves, with stalks removed, were washed in neutral EDTA (1%) solution. The cedar leaves were dried for 12 hours at 100°C, and then 1 g of the dry matter was dry ashed at 450°C for 12 hours. After dry ashing, the ash was extracted with 10 ml 10% nitric acid, and subsequently analyzed with a Hitachi 207 atomic absorption spectrophotometer. In the control area the average cadmium concentration (dry weight) in soil was 1.5 μg/g (S.D. = 1.0). In the cedar leaves from the control area the concentration was 1.2 (±1.5) μg/g. The corresponding values for the polluted area were 12.9 μg/g±16.2 in soil and 26.0±23.1 μg Cd/g in "shida" leaves. There was thus a 20-fold increase in cadmium content of "shida" leaves as compared to a 10-fold increase in cadmium content in soil. According to Ishizawa et al., 1974, the iron concentration in soil and "shida" bush was not different between the control area and the polluted area and the manganese concentration in the polluted area was about half of that in the control area. There was no significant difference in the calcium concentration in soil and leaves between the two areas. Compared to the control area, the polluted area showed increases of 15-fold for zinc in soil versus 10-fold for zinc in "shida" leaves, 60-fold for copper in soil versus 2-fold for copper in "shida" leaves, and 6-fold for lead in soil versus 0-fold for lead in "shida" leaves. From these data Ishizawa et al. drew the conclusion that cadmium and zinc accumulate to the greatest degree in "shida" leaves, making "shida" leaves a suitable indicator of cadmium pollution. Ishizawa et al., 1974, also presented data on the heavy metal concentrations in 21 samples of mosses taken close to the mine in the polluted area. Cadmium concentration varied between 0.7 and 15 μg/g dry weight.

Kobayashi et al., 1973, reported cadmium, lead, and zinc concentrations of soil samples taken at various distances and in various directions from the zinc smeltery in Annaka. Some of their data have been referred to in CITE, 2nd ed., as unpublished data by Kobayashi.

One aspect concerning the air pollution around Annaka not mentioned in CITE, 2nd ed., is the correlation between the concentrations of these metals. A very good agreement especially for zinc was shown, which may indicate that zinc concentrations in soil may be used as an indicator of cadmium concentrations in large-scale environmental studies around point sources where both zinc and cadmium are emitted. Zinc concentrations in this study were on the average 57 times higher than cadmium concentrations and may therefore be easier to analyze.

3.1.3 Cadmium in food

New data are now available on cadmium in food. These data include new values from some of the countries from which data were furnished in CITE, 2nd ed., as well as some countries from which values were previously not known.

In Austria Woidich and Pfannhauser, 1974, determined cadmium in food items by a dithizone method and an atomic absorption method after extraction with APDC-MIBK. In Table 3:3 the data are shown. The concentrations of cadmium are extremely high in the cereals, oats having higher cadmium concentrations than even mussels. On the other hand, the data on cadmium in pork meat and pork kidney agree relatively well with other data, such as those of Hecht, Mirna and Schramel, 1973, who found a mean concentration of 0.005 μg/g wet weight in 60 samples of pork meat.

In U.K. an extensive survey of cadmium in food was made (Ministry of Agriculture, Fisheries and Food, 1973). Food was obtained from different towns and cities throughout the U.K., as part of a Total Diet Study. The foodstuffs were arranged into eight groups, each with similar types of food, and made into composite samples. When appropriate the food was also prepared for consumption and the water used analyzed for cadmium. Food items were also analyzed individually.

Cadmium was determined by atomic absorption spectrophotometry in different laboratories. Samples were either wet ashed or dry ashed. Some laboratories extracted with APDC and 4-methylpentane--2-ketone. The detection limit was reported to be 10 ng/g, except for water and milk, for which it was 5 and 1 ng/g respectively.

Altogether 4000 results were obtained. It was estimated that the cadmium concentration in the average diet in U.K. is probably between 10 and 20 ng/g. In individual food items the concentrations were found to be generally between 10 and 40 ng/g, the exceptions being kidney, fresh carrots and potatoes, the latter (19 samples) having an average of 80 µg/g (range 10-170 µg/g). This value is surprisingly high, since the average for composite root vegetables (where potatoes must have been included) in the total diet showed an average of less than 20 ng/g. The same data on cadmium in potatoes are found in a paper by Thomas, Roughan and Watters, 1972, where it is stated that the method used was extraction with APDC and a ketone. Lamb meat was found to have the same average concentration as lamb liver, 20 ng/g, which does not fit with the fact that muscle concentrations are considerably less than liver concentrations as shown in CITE, 2nd ed.

The mean for all milk samples was <2 ng/g, confirming that the cadmium content of milk is very low.

Whereas in the total diet study the average of composite fish samples was <20 ng/g (<10-50) a separate study on fish meat showed higher values. The range was from <30 to 750 ng/g.

In a special study on crabmeat, the average concentrations of cadmium in dark meat from different areas around the U.K. varied between 0.78-21 µg/g wet weight, whereas the corresponding averages for white meat were from <0.08 to 0.33. Shellfish other than crabs also contained relatively high concentrations of cadmium.

This survey gives valuable information and confirms that the levels of cadmium are generally low in most foods. Since different laboratories analyzed the samples and interlaboratory comparisons are not reported, the results must be regarded as only approximate. There are some obvious inconsistencies between e.g. data on fish from two studies.

Thomas, Roughan and Watters, 1973, reported on cadmium in canned fruit and vegetables consumed in U.K. Cadmium was determined by atomic absorption after extraction with APDC and 4-methylheptaneketone. Most fruits contained around or less than 10 ng/g, of canned vegetables, only spinach having a relatively high concentration, 80 ng/g (range 30-180 ng/g).

In Canada, Kirkpatrick and Coffin, 1973, determined cadmium by atomic absorption with background correction in 190 cured meat samples. In Table 3:4 it is seen that the average varied between <10 and 30 ng/g in all groups. These are relatively low concentrations, but in view of the difficulties in determining cadmium in meat, they might well be lower.

Horwitz and van der Linden, 1974, determined cadmium in 5 brands of tea and 7 coffees, the average for both being 30 ng/g. It was calculated that a cup of coffee or tea contained from 50 to 220 ng cadmium.

Beckman et al., 1974, determined cadmium by atomic absorption with background correction in liver and kidneys from cattle, pigs, sheep and horses in Sweden. In Table 3:5 it is seen that in kidneys the cadmium concentrations are relatively high even in young animals, and the liver concentrations are generally above what is found in other foods. In horse kidney and liver cadmium concentrations were quite high.

A special study on baby foods was done in U.K. by Snodin, 1973. Cadmium was determined by atomic absorption after extraction into DDDC-MIBK (DDDC = diethyl ammonium diethyldithiocarbamate). In Table 3:6 the results are presented. It should be noted that canned and bottled food are consumed as they are, whereas some of the dried foods, e.g. milk, have to be reconstituted before consumption. These data show higher levels in baby food than those presented in the above mentioned, extensive U.K. study. Further confirmation of the low levels of cadmium in milk has been given by Cornell and Pallansch, 1973. By using a pulse polarographic technique they found average concentrations of 0.12, 0.24 and 0.23 μg/g by repeated sampling of milk from 3 cows.

Sugiyama, Ishizawa and Yoshida, 1974, studied the heavy metal content of various organs of Japanese squid: liver muscle, testis, "sexual gland", ovarium, "squid uterus", and contents of the stomach. Forty immature and mature squid were analyzed. The average cadmium concentration (dry weight) in liver was 160±114 (S.D.) μg/g, and the zinc concentration 121±64 μg/g. In meat the concentration of cadmium was 1.9±1.0 μg/g, and the zinc concentration 64±8 μg/g. On the other hand in testis, ovarium and "sexual glands" the zinc concentrations were about 60 times higher than the cadmium concentration. Cadmium in testis was e.g. 2.3±1.9 μg/g, whereas the zinc concentration was 135±31 μg/g. The wet weight concentration of metals is not presented in the paper.

Enomoto and Uchida, 1973, reported data on cadmium contents of various seafoods from the Ariake Sea in southern Japan. Parts of the area are polluted by e.g. the zinc-refineries in Omuta but other parts may be basically non-polluted by cadmium. Atomic absorption after wet ashing and extraction was used for cadmium analysis. The muscle of one type of fish (sarubou) caught at 4 different places contained 1.01-6.29 μg Cd/g dry weight (0.2-1.3 μg/g wet weight) and the corresponding internal organs contained 11.5-24.7 μg Cd/g dry weight (0.23-4.9 μg/g wet weight).

In muscles of various fish bought at the market in Saga, a city in the vicinity of the Ariake Sea, the concentrations were 0.04-0.26 μg Cd/g dry weight (0.008-0.05 μg/g wet weight). Canned seafoods were also analyzed giving e.g. an average of 0.15 μg Cd/g dry weight in 8 samples of mackerel from a non-polluted area in San-in area, an average of 5.0 μg Cd/g dry weight in 15 samples of oysters from Hiroshima Prefecture and an average of 3.9 μg Cd/g dry weight in 10 canned samples of "Akagai" from Ariake Sea.

Akita et al., 1974, studied the transfer of cadmium from rice and various vegetables to water solutions. Dried samples of polished rice (original cadmium concentration 2 μg/g dry weight) were kept in water for 24 hours at 25°C, 30°C and 50°C. The cadmium lost to water was 3-5 %. When leafy green vegetables were boiled for 10 minutes 34-61 % of their original cadmium contents (0.04-1.58 μg/g) were lost to water while root crops (sweet potato, etc.) lost between 6 and 45% (0.33-1.55 μg/g). Cadmium was analyzed with flame atomic absorption after wet ashing and APDC/MIBK-extraction.

3.1.4. Cadmium in cigarettes

In CITE, 2nd ed., it was concluded that a cigarette contains from 1 to 2 μg of cadmium and that about 10 % of that amount may be inhaled.

Unterhalt and Pindur, 1972, determined the cadmium content of 4 cigarettes by atomic absorption and found a mean amount of 1.44 μg per cigarette. The mainstream smoke from 10 cigarettes was collected and an average of 0.33 μg was said to be contained in that fraction. The technique is not described and no certain conclusions can be drawn, since standardized techniques should be used for such studies.

Since smoking will cause significant increases in body burden of cadmium it is of interest to know whether there have been changes in the cadmium content of cigarettes with time. Such a study has been performed by Linnman, Lind and Kjellström (to be published). Eighteen brands of cigarettes produced in the years 1918-1968 were analyzed. In Fig. 3:2 it is seen that with one exception the concentrations are similar to those found today, with no tendency toward an increase.

Lettuce leaf cigarettes, which are used as a substitute for tobacco, were examined by Elia, Menden and Petering, 1973. The average cadmium content was 1.39 μg per cigarette. These cigarettes were smoked in a smoking apparatus using a standardized procedure. In the mainstream only 0.01-0.02 μg was found, which is less than what has been found in ordinary cigarettes. On the other hand more cadmium was emitted into the air via the sidestream.

3.1.5. Changes in cadmium concentrations in food over time

A question of importance is whether or not the present daily cadmium intake in a given area is different from the cadmium intake in the same area years ago. Due to the very long biological half-time of cadmium in the critical organ, the kidney (CITE, 2nd ed.), such data are necessary for a meaningful evaluation of the dose. Due to the problems of analyzing cadmium in a reproducible way at the low concentrations in common foodstuffs (see Chapter 2), it is doubtful as to whether studies performed at different times by different laboratories can be compared. One good alternative might be to analyze cadmium in old foodstuffs, which for one reason or another have been preserved to the present day. Kjellström et al., in press, analyzed the cadmium content in Swedish wheat samples harvested in the same geographical area, Uppsala and environs: 31 samples of spring wheat from 1917-1972 and 45 samples of fall wheat from 1916-1972. Data on meteorological factors and fertilizers were available from past records. Some studies on barley were also performed.

The samples were treated in a completely blind fashion (duplicate analysis) according to the method by Kjellström et al., 1974b. There was a wide scatter between the individual values (Figures 3:3 and 3:4), but for the whole 50-year period, there was a statistically significant increase in the average cadmium concentration in fall wheat. In spring wheat such an increase occurred between 1916 and some time in the 1950's. There was a very strong within-year correlation between the cadmium concentrations in different genotypes of wheat and between wheat and barley harvested in the same area. This suggests that some meteorological, agricultural or pollution factor, or a combination of such factors, related to the individual year could explain the variation in the cadmium concentration. Statistical analysis did not reveal any correlation between cadmium concentration and any of the following factors: yield, average summer temperature, average summer rain, type or amount of fertilizer applied. The zinc concentration in grains did not show the same pattern, and the ratio of cadmium to zinc in wheat increased in the same fashion as cadmium concentration itself.

Kjellström et al., in press, concluded that the increase in average Cd-concentration in fall wheat may be caused by accumulated effects of air pollution and fertilizers.

3.2 PRESENT DAILY INTAKE OF CADMIUM

In CITE, 2nd ed., it was concluded that the daily intake of cadmium in non-polluted areas probably was around 50 μg.

In the large study in U.K. (see 3.1.3) it was estimated that the average intake there was between 15 and 30 μg per day. In another study from U.K. an average intake of 64 μg was estimated (Hamilton and Minski, 1973).

Guthrie, 1973, reported that the average intake of cadmium by 11 women in New Zealand was 48 (21-104) μg per day. The method was atomic absorption without background correction. The author later corrected these values for background interference and reported in an addendum that the intake was 21 (7-76) μg.

Wester, 1974, in a metabolic study, determined cadmium intake by analyzing duplicates of the daily diet in a Swedish hospital. Mean values for 4 subjects during two 5-day periods were 10-11 11-12, 5.1-6.0 and 9.6-13 μg. The method was neutron activation. The corresponding values in feces were 9.8-7.2, 13-13, 6.5-4.8 and 8.9-3.4, thus showing, except in one observation, good agreement with the intake values.

The daily intake of cadmium in Japan has been discussed in a review by Yokohashi, Suzuki and Matsubara, 1973. There are unpublished data by Ro, Suzuki and Tanaka, regarding cadmium in food, beverages, feces and urine of one 38-year old male subject.

Samples had been taken every day for 30 days, showing an average daily cadmium content in food and drinks of 45.7 μg, in feces of 32.6 μg, and in urine 4.2 μg. The analytical method was based on atomic absorption, but no further details were given. The food and feces data indicate an absorption of about 30% of alimentary cadmium, which is a figure much higher than earlier reported (CITE, 2nd ed.). This may be explained by analytical errors. Apart from this, these data resemble the data on the Japanese cadmium intake previously compiled (CITE, 2nd ed.).

Tati, Katagiri and Kawai, to be published, determined the fecal output of cadmium for 5 days in 7 Japanese students. The average output varied between 41.1 and 79.4 μg/day and assuming 5 % absorption the daily intake was estimated to be from 43.3 to 83.6 μg per day.

Data on fecal concentrations of cadmium are given by Johnsson et al., 1974. They found in 6 different population groups (n = 27-41) in Houston, Texas, mean values varying from 0.19-0.30 μg/g. Assuming the average fecal daily weight to be as high as 200 g this would correspond to a daily output of 38-60 μg, which corresponds to a daily intake of 40-63 μg. This is a very rough estimate based on a high weight of the daily feces, and the true values are probably lower.

3.3 CONCLUSIONS

New data confirm that the "normal" levels of cadmium in air are less than 1 ng/m^3 and that in industrialized areas considerably higher levels may be found. Further studies on uptake of cadmium in plants confirm that increasing the pH of soil reduces the uptake.

New data on cadmium in food confirm that the levels generally are low in most foods, but that internal organs may contain high concentrations.

A change in the cadmium content of cigarettes with time has not been demonstrated. Studies on wheat indicate a slow but significant increase in cadmium concentrations with time in wheat grains in a Swedish area.

Earlier estimates of the daily intake of cadmium in non-polluted areas in Japan have been confirmed, i.e. around 50 μg, whereas new data from U.K., Sweden and New Zealand indicate that the intake there may be 10-30 μg.

TABLE 3:1

EFFECT OF CADMIUM AND PHOSPHATE APPLICATION ON CADMIUM CONTENTS IN WHEAT (From Koshino, 1973).

Application rate			Cd content (μg/g air-dry basis)						Cd uptake (μg/pot)				
P_2O_5	Cd	Others	3/20	4/30	at the harvest (6/17)				at the harvest (6/17)				
(g)	(mg)		Tops	Tops	Root	Straw	Grain	Abortive grain	Root	Straw	Grain	Whole plant	Tops
0.5	0		0.2	0.1	1.1	0.4	0.16	0.0	4	6	1.0	11	7
	100		25	16	79	9.8	2.7		142	45	1.9	189	47
1.5	0		1.2	0.3	1.0	0.6	0.12	0.15	5	19	2.2	26	21
	30		34	8.1	29	5.2	1.6	1.9	209	176	32	418	210
	100		40	16	78	10.8	2.3	2.4	343	266	35	645	302
	250		34	8.5	81	8.0	2.2		97	30	4.8	132	35
	500		34	23	400	18.6	2.4		120	19	0.1	139	19
4.5	0		1.7	0.4	1.8	0.4	0.16	0.11	20	20	6.2	46	26
	100		43	14	55	10.2	2.9	3.3	401	409	108	920	519
1.5	0	limed	0.6	0.1	2.8	0.4	0.13	0.14	20	15	3.0	38	18
	100	limed	16	5.6	31	3.8	1.3	1.5	279	147	29	460	181
1.5	0	granular	0.4	0.3	0.6	0.4	0.10	0.3	3	15	3.1	21	18
	30	granular	13	5.4	14	4.5	1.0	1.8	63	136	21	220	157

TABLE 3:2

ANALYSIS OF THE SOIL AFTER CROPPING WITH WHEAT (OVEN-DRY BASIS) (From Koshino, 1973).

P_2O_5	Cd	pH	0.1 N HCl soluble (μg/g)**			
(g)	(mg)	(KCl)	Cd	Zn	Mn	Cu
0.5	0	5.5	0.2	35	26	15
	100	5.4	32.5	37	24	15
1.5	0	5.5	0.4	26	24	16
	30	5.5	17.4	35	32	16
	100	5.5	34.5	32	28	17
	250	5.7	120	46	29	16
	500	5.7	170	24	20	15
4.5	0	5.4	0.4	32	27	15
	100	5.5	32.5	47	40	15
1.5	0*	6.3	0.4		40	15
	100*	6.7	26.3	34	29	10

*Limed with 50 g $CaCO_3$.

**Soil/solvent ratio is 1/5 for Cd and 1/10 for other metals.

TABLE 3:3

CADMIUM CONTENT IN FOODSTUFFS (From Woidich and Pfannhauser, 1974).

Type	No.	Cadmium µg/g wet weight Range	Mean
Corn	3	0.59-1.05	
Wheat	13	0.006-1.05	0.24
Oats	4	1.1-1.93	
Barley	4	0.05-0.17	
Rye	4	0.125-0.27	
Mussle	39	0.05-1.74	0.31
Tuna-fish	13	<0.05-0.97	0.14
Sardine	27	<0.05-1.00	0.23
Fish (except tuna-fish and sardine)	65	<0.05-1.46	0.13
Pork kidney	15	0.01-0.41	0.15
Pork	15	0.008-0.025	0.014

TABLE 3:4

CADMIUM CONCENTRATIONS IN CURED MEATS (FRESH WEIGHT BASIS) (From Kirkpatrick and Coffin, 1973).

Meat type*	No. of samples	Cd (µg/g)	
		Range	Mean
A-1	15	<0.01-0.22	0.02
A-3	17	<0.01-0.04	0.01
A-4	20	<0.01-0.07	0.03
A-5	20	<0.01-0.04	0.02
A-6	25	<0.01-0.05	0.02
A-7	15	<0.01-0.09	0.03
B-1	18	<0.01-0.06	<0.01
B-2	23	0.01-0.06	0.03
B-3	14	<0.01-0.06	0.02
B-4	23	<0.01-0.08	0.02
Total	190	<0.01-0.22	0.02

* Ready-to-eat cured meat products canned (in metallic containers) cured meats

A-1 = Shelf-stable

Packaged (non-metallic) cured meats

A-3 = Wieners - including smoked and unsmoked
A-4 = Bologna
A-5 = Meat loaves (luncheon meat slices, sliced ham, mock chicken, macaroni and cheese, luncheon meat slices, etc.)
A-6 = Salami, including various sausage products such as European type sausages.

Unpacked cured meat products

A-7 = Pastrami, smoked meat, dried uncooked (Westphalian) ham etc.

Cured meat products requiring further cooking by the consumer

B-1 = Bacon
B-2 = Hams
B-3 = Picnics (shoulders)
B-4 = Corned beef products

TABLE 3:5

CADMIUM CONTENTS IN KIDNEY AND LIVER OF SOME FOOD-PRODUCING MAMMALS (From Beckman et al., 1974).

Species	No.	Age, years		Cadmium, µg/g Kidney		Liver	
		m	r	m	r	m	r
Cattle	9	0.21	(0.12-0.33)	0.25	(<0.02-0.75)	0.06	(<0.02-0.17)
Cattle	28	2.9	(0.42-12)	0.62	(0.11-3.2)	0.11	(0.03-0.33)
Pig	12	0.47	(0.37-0.58)	1.02	(0.16-3.1)	0.12	(0.04-0.24)
Sheep	16	1.06	(0.33-4.00)	0.43	(<0.02-3.5)	0.34	(<0.02-2.8)
Horse	13	12	(1.0-26)	31	(4.3-90)	2.6	(0.15-6.7)
Foal	1	0.08		0.04		0.04	

m = mean
r = range

TABLE 3:6

CADMIUM CONTENTS OF SOME TYPES OF BABY FOOD (From Snodin, 1973).

Food	No. of samples	Cadmium µg/g (mean)
Dried milk	8	0.020
Dried Cereal	15	0.035
Canned Foods	7	0.036
Bottled Foods	7	0.026

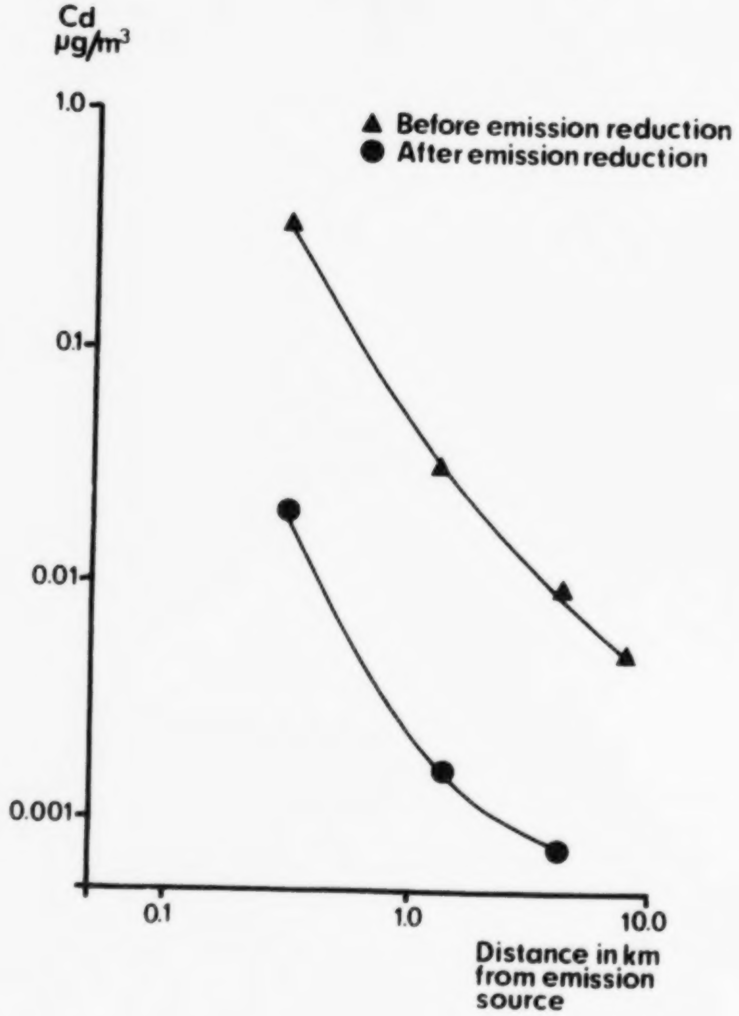

FIGURE 3:1 Average cadmium concentrations in air at different distances from a factory producing copper-cadmium alloys. Continuous sampling was performed during May-December 1971, and May-December 1972. Each sample represented 24 to 72 hours. In December 1971 emission-reducing equipment was installed (From Lind and Piscator, unpublished data).

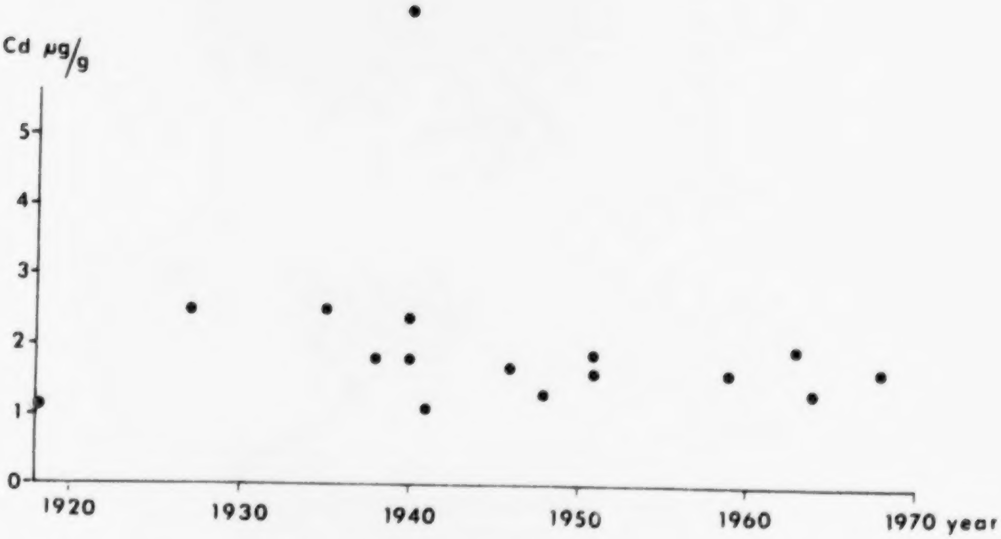

FIGURE 3:2 Cadmium concentration in cigarettes of different ages (µg/g dry weight). (From Linnman, Lind and Kjellström, 1974).

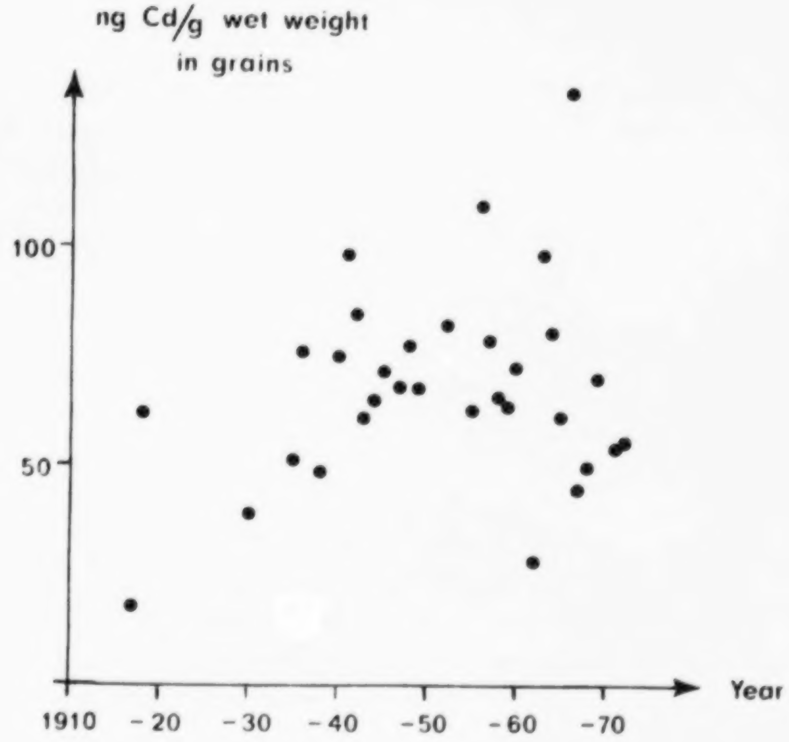

FIGURE 3:3 Cadmium concentration in spring-wheat from Uppsala, Sweden. (From Kjellström et al., in press).

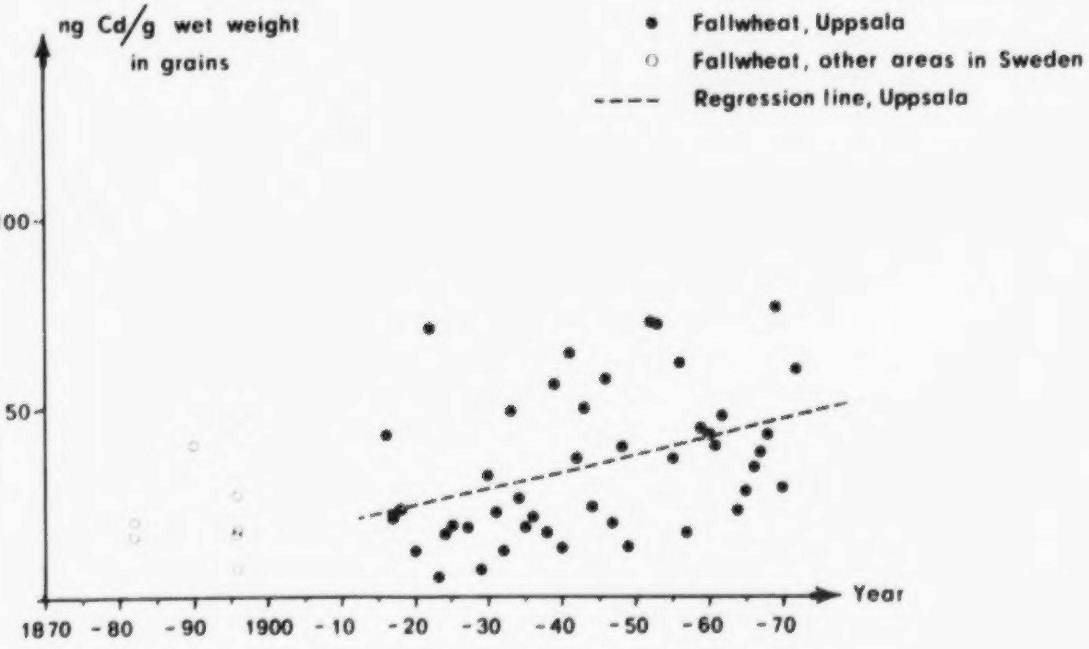

FIGURE 3:4 Cadmium concentration in fall-wheat from Uppsala and other areas in Sweden. (From Kjellström et al., in press).

Chapter 4. METABOLISM

4.1 UPTAKE AND ABSORPTION

In CITE, 2nd ed., it was concluded that cadmium will mainly be absorbed from the lungs and the gastrointestinal tract, even if under certain circumstances soluble cadmium compounds may be absorbed from the skin. A recent report (Kimura and Otaki, 1972) on percutaneous absorption of cadmium chloride in mice and rabbits after repeated applications in water solution or in ointment confirmed earlier results, e.g. cadmium may also enter via the skin, about 0.5% of the applied amount being deposited in liver and kidneys. There are no data on absorption after exposure via skin in human beings.

4.1.1 Respiratory deposition, clearance,and absorption

In CITE, 2nd ed., it was concluded that inhaled cadmium is absorbed and retained to a considerable degree. Human data were scarce, but it was estimated that up to 50% of inhaled cadmium fumes may be absorbed. Animal data indicated an absorption of 10 - 40%.

There are no human studies available primarily designed for determination of the pulmonary handling of cadmium, but evidence presented in sections 4.3.2 and 4.3.3 indicates that cadmium from cigarettes must be absorbed to a high degree.

One animal experiment has been reported. Moore et al., 1973, studied the fate of radioactive cadmium after different administration routes. In inhalation experiments rats (numbers not given) were exposed to a ^{115m}Cd chloride aerosol at a concentration of 1,800 μg Cd/m^3. Exposure time was four hours. The mean diameter was about 0.5 micron. Measurement of ^{115m}Cd in the lungs of animals sacrificed immediately after cessation of exposure indicated a retention of 9.7% of the total inhaled ^{115m}Cd (using a minute volume of 0.1 liter for calculating the amount of inhaled cadmium). Unfortunately no data are given on cadmium concentrations in liver and kidneys. About 60% of the cadmium in the body was at that time found in the gastrointestinal tract.

The biological half-time after the first rapid initial clearance during the first week was about 200 days as measured by whole body counting. This half-time was about the same as was found in other exposure routes as seen in Figure 4:1.

4.1.2 Gastrointestinal absorption

4.1.2.1 Gastrointestinal absorption in animals

In CITE, 2nd ed., it was concluded that the gastrointestinal absorption rate is usually about 2% in animal experiments. The absorption should be increased considerably by low calcium or low protein intake.

Suzuki, Taguchi and Yokohashi, 1974, gave ^{109}Cd by stomach tube as a single exposure to two Macaca irus monkeys, who weighed 3.60 and 3.13 kg. The night before the experiments the monkeys were thirsting. In the morning 105 μCi carrier free $^{109}CdCl_2$ was given to monkey A after which he was allowed to drink and eat freely. Monkey B received 57 μCi $^{109}CdCl_2$ in the same fashion, but in this case mixed with non-radioactive cadmium chloride, such that 1 mg of cadmium was given in 10 ml solution (concentration 100 μg Cd/g). Monkey A was killed after 19 days, and monkey B after 25 days, and the cadmium content in the various organs was measured with scintillation counter. During the experiment feces and urine were collected each day and cadmium content measured.

In Figure 4:2 the distribution of the dose given to monkey A is depicted. It is seen that 12 % of the dose is retained after 19 days, and the larger part of this retention is still in the gastrointestinal tract. The retention in the rest of the body is 5.3 %; for the other monkey (B) this figure was 6.35 %. Using the whole body cadmium concentration as a reference, Suzuki, Taguchi and Yokohashi underlined the relative concentration of cadmium in the epithelium of the small intestine. The concentration is about 33 times higher than the average body concentration, and in the duodenum it is more that 50 times higher. The only other organ where such high cadmium concentrations are found is the kidney, where the cadmium concentration is about 20 times higher than the average concentration in total body. The initial high concentration in duodenal mucosa has also been observed by Fox, 1974.

Suzuki and Taguchi, 1972, had earlier reported a similar experiment on another Macaca irus monkey. The retention rate in whole body except for the gastrointestinal tract was 0.65 %. The new data by Suzuki, Taguchi and Yokohashi, 1974, indicate that the uptake of cadmium from the gastrointestinal tract is a slow process, taking several days, or even weeks. The daily fecal excretion has 3 phases each following an exponential decrease. During the first week of the experiment the half-time is 1 day, during the second week about 2 days, and after the second week about 41 days. In the 1974 experiment, absorption rate is similar between the two monkeys even though one received carrier free cadmium and the other received cadmium with carrier.

Moore et al., 1973, gave rats (number not stated) single oral doses of $^{115m}CdCl_2$, 50 µCi by stomach tube. After the initial rapid elimination about 3 % of the dose was retained after 4 days and 2.3 % after 10 days, as shown in Figure 4:1, indicating that absorption must have been of that magnitude.

A long-term study was performed by Ogawa et al., 1973. For 30 days they gave two groups of mice radioactive cadmium in drinking water. One group was given 50 µCi/l of carrier free $^{115m}CdCl_2$ solution (containing 0.35 µg/g of cadmium) and the second group was given 50 µCi/l of carrier containing $^{115m}CdCl_2$ in water (containing 146 µg/g of cadmium). The animals were allowed to drink ad libitum. Whole body radioactivity was measured at one week intervals up to day 31. It was found that whole body retention of ^{115m}Cd was significantly less in the carrier group. It was also calculated that the average cadmium uptake for 30 days was 0.56 µg/day in the first group and 100 µg/day in the second group. This gives a ratio of about 200 meaning that the high exposure group retained only about 50 % of what could be expected were the retention proportionate to the exposure. The authors concluded that absorption of cadmium was lower at the higher exposure level. When liver and kidney are studied alone the corresponding figures will be 140 and 40 % respectively. It is not clear from the description of the experiment whether the daily consumption of water was similar in the respective groups. Since the high dose caused toxic symptoms, the intake might have been less in that group.

Matsusaka et al., 1972, studied whole body retention of $^{115m}CdCl_2$ after single and repeated peroral infusions via gastric tube to adult mice and young mice of the age 7, 14 and 21 days. Cadmium retentions after single exposure (Figure 4:3) are higher in the younger mice (five animals in each group). As seen in the same figure the retention of $CdCl_2$ injected intravenously is almost 100%. Because the retention pattern of the 7-day old mice differed from the others Matsusaka et al. repeated the experiment on 20 such mice and killed them 1, 5, 10 and 20 days after single exposure. The cadmium content of the intestines was deducted from whole body and it could be shown that the actual gastrointestinal absorption in these mice was 8.1 - 9.2% 1, 5, 10, and 20 days after exposure. Repeated exposure of adult mice and 7-day old mice during 10 days showed that the excretion of infused cadmium was much slower from day 7 - 15 after birth. The data indicate that gastrointestinal handling of cadmium in 7-day old mice may be the cause of the different retention patterns. Whether the differences in total retention between different age groups also are caused by this factor is not possible to say.

A long-term study on lambs has been performed by Doyle et al., 1974. Five groups with six lambs in each were fed cadmium-containing diets for 191 days. The diets contained 0, 5, 15, 30 and 60 µg/g cadmium. After 163 days the animals were transferred to metabolism stalls for 21 days subsequent to which a 7-day absorption study was performed. Cadmium intake and absorption were determined from food and feces data. Only absorption data on the control group and the group given 60 µg Cd/g of diet are given in the paper. The control lambs had a mean intake of cadmium of 180 µg/day and the absorption was calculated to be 11%. The intake in the exposed group was 119 mg/day and the absorption was calculated to be 5%. Since the urinary excretion of cadmium in controls was reported to be higher (45 µg/lamb/day) than in the highest exposure group (36 µg/lamb/day) there is reason to doubt the reliability of the analytical methods.

The influence of cholecalciferol (vitamin D_3) on the absorption of cadmium has been studied by Cousins and Feldman, 1973. In one experiment male chicks were given a vitamin D deficient diet containing 1 % calcium for 28 days. On the 27 th day of life one half of the group received 2000 I.U. of cholecalciferol orally. 18 hours later each chick received 5 µCi of ^{109}Cd orally. After a 24-hour fast the chicks were killed. The cadmium content of liver and kidney of the chicks given vitamin D was higher than in non-treated animals, but the difference was not significant. In another experiment chicks were given the vitamin D deficient diet for 23 days, and then for 4 days half of the group received 600 I.U. of cholecalciferol per kg of diet. On the 5th day all chicks received 5 µCi of ^{109}Cd orally. After a 24-hour fast the chicks were killed.

This experiment likewise revealed no difference between the groups with regard to liver and kidney levels of cadmium. It was concluded that vitamin D does not have a direct influence on the uptake of oral cadmium into the liver and kidney. The results from this study are thus in contrast to earlier work presented in CITE 2nd ed., where it was mentioned that Worker and Migicovsky, 1961, had shown that vitamin D had an effect on cadmium metabolism. The difference is, as discussed by Cousins and Feldman, that in the present study the animals were receiving a sufficient amount of calcium, whereas in the study by Worker and Migicovsky a low calcium diet was given. It has indeed been documented in CITE 2nd ed., that calcium deficiency causes an increase in the absorption of cadmium.

Long-term studies on rats have been reported by Ishizaki, 1972. Two basic diets were employed, one with high protein - high calcium and one with low protein - low calcium content. Cadmium concentrations in the diets were varied by adding rice with high and low cadmium content. Eleven male and eleven female groups were used and after 28 weeks cadmium was analyzed in liver and kidneys. It was found that rats on low calcium - low protein diets accumulated more cadmium than rats on high calcium - high protein diet. Regardless of diet, it was seen that females accumulated more cadmium than males. The mean retention after 28 weeks was 0.47 % in 4 low protein - low calcium groups receiving between 0.9 and 8.55

μg Cd/g of diet , and 0.28 % in 2 groups on high protein - high calcium diet and with 2 and 2.57 μg Cd/g of diet. These findings support the findings discussed in earlier reports. In a brief note, it was reported (Washko and Cousins, 1974) that calcium deficiency caused higher retention of orally given cadmium in male rats. Pribble and Weswig, 1973, compared the uptake of cadmium in water and food respectively. Weanling rats, 10 of each sex in three groups, were given a normal diet, a diet with added cadmium chloride 5 μg/g food, and cadmium in water, 5 μg/g for 18 months. The intake of water per weight was 50 % higher than the intake of food. The accumulation in liver and kidney was about 2.5 times higher in animals given cadmium in water and the difference in intake could not explain this difference. The authors postulated that cadmium in water could be more easily accessible than cadmium bound to organic molecules in the food.

4.1.2.2 In human beings

In CITE 2nd ed., it was stated that ingested cadmium was absorbed to about 6% in human beings, but that low calcium intakes might well cause higher absorption.

Yamagata, Iwashima and Nagai, 1974, studied the gastrointestinal uptake of radioactive cadmium in a 53-year old male. Rice was grown in pots to which ^{115m}Cd was added and at the time of the experiment 20 g of this rice was available, containing a total of 1.5 μCi ^{115m}Cd. The rice was mixed with regular polished rice to make a total amount of 100 g, which was eaten at one time and whole body counting was performed during 144 hours. Figure 4:4 shows the decrease in whole body content of radioactive cadmium. Yamagata, Iwashima and Nagai also collected 24-hour samples of feces and urine from the subject during the first three days of the experiment. In feces the excretion was 76.2, 15.8 and 1.2 % of the given dose, and in urine 0.7, 0.0 and 0.9 %. These analyses were also performed by measuring radioactivity. The total excretion during three days was 95 %, a figure which fits well with the retention value in Figure 4:4. After six days the retention was 4.4 % of the given dose. The authors concluded that the gastrointestinal absorption in human beings may well be 5-7 %.

Robinson et al., 1973, performed a metabolic balance study in 4 human beings aged 19-25 years. The duration of the study was 27 days, divided into three 6-day experimental periods, a 6-day preliminary period and a 3-day post-period. The diet was invariably meat loaf, ice cream, tea and coffee. During the different periods a constant amount of food was given each day, and it was thus possible to calculate the daily intake for each subject. The mean daily intake of cadmium in the 4 subjects was estimated to be 60-92 µg/day. Daily collections of urine and feces were made. For analysis of cadmium atomic absorption was used, cadmium in urine being determined by direct method with standard additions of cadmium and cadmium in food and feces being determined in acid digest. Background correction was not utilized. Urinary excretion of cadmium was found to be from 14-54 µg/day according to the method applied, and it was estimated that between 36 and 41 % of dietary cadmium was excreted in urine. The fecal excretion was calculated to vary between 42 and 74 % of the intake in the 4 subjects. Since data from other human studies have clearly indicated that less than 10 % of ingested cadmium is generally absorbed the data from this study are obviously quite erroneous. The methods chosen are not of sufficient accuracy for determination of cadmium in biological material.

4.1.3 Placental transfer

Ishizu et al., 1973, gave pregnant mice single subcutaneous injections of 2.5 mg Cd/kg as cadmium chloride on the 7th day of gestation. In placenta the cadmium concentrations were about 10 times higher than in the placenta of controls, whereas cadmium concentrations in the fetuses were similar, being about 0.003 µg/g. Mean concentrations of 17 µg/g and 0.19 µg were detected in the liver and placenta respectively in the cadmium-injected mothers.

These data indicate that the subcutaneous injection of relatively large doses of cadmium does not cause any significant accumulation of cadmium in the fetus. This is in contrast to some earlier animal studies, discussed in CITE, 2nd ed., where it was shown that exposure to large amounts of cadmium via intravenous injections could cause uptake in the fetus.

The dose given was shown to cause malformations, possibly due to an indirect effect of cadmium rather than to a direct one. Interference with zinc metabolism might well be responsible.

4.1.4 Conclusions

New animal data on absorption after inhalation give further evidence that cadmium is retained for long times after respiratory exposure. Additional proof has been obtained that low calcium intakes cause increases in gastrointestinal absorption of cadmium. There is an indication that differences exist between absorption rate for cadmium compounds in water and foodstuffs, a problem that should be further studied. New data on gastrointestinal absorption in a human being confirm that about 6 % of ingested cadmium is absorbed.

4.2 TRANSPORT, DISTRIBUTION AND EXCRETION OF CADMIUM IN ANIMALS

4.2.1 Uptake to and clearance from blood

In CITE 2nd ed., it was concluded that after a single injection, cadmium will initially be in the plasma, but during the first 24 hours after injection a rapid clearance from plasma takes place so that eventually the concentration in the cells will exceed that in the plasma. After repeated exposure cadmium will mainly be found in the blood cells, bound to proteins, such as metallothionein.

Yoshikawa, 1973, studied the distribution of cadmium in rats. Twenty rats were given 0.6 mg of cadmium/kg body weight intraperitoneally and 24 hours later that group and another untreated group of 20 rats received intraperitoneally 3 mg cadmium/kg body weight. Four rats in each group were killed 2, 6, 24, 72 and 168 hours after the last injection. Cadmium was determined in red cells and in plasma. Figure 4:5 shows a tendency to falling levels in red cells in non-pretreated animals during the first six hours, but later on an increase, whereas in pretreated animals such an initial drop in cadmium concentrations in the cells is not seen.

Plasma levels 6 hours after injection were considerably higher in the animals without pretreatment than in pretreated animals. The results in animals without pretreatment are in accord with earlier findings presented in CITE 2nd ed., where it was assumed that the second increase in red cell concentrations had to do with the formation of metallothionein, which had been found in red cells 24 hours after injection. These new data support the assumption that the formation of metallothionein is connected with the uptake of cadmium in the red cells.

Cadmium in blood was followed by Matsubara-Khan, 1974, for 128 days after a single subcutaneous injection of 1.7 μCi of ^{109}Cd as cadmium chloride in 40 male and 40 female mice. Figure 4:6 shows the cadmium levels in blood at different times after injection. The biological half-time in blood was calculated by the author to be 29 days.

4.2.2 Tissue distribution and retention

In CITE 2nd ed., it was concluded that cadmium was mainly stored in liver and kidneys. The distribution between these two organs will depend on route of administration and dose. Thus long-term low level oral exposure will cause high concentrations in kidney and relatively low concentrations in liver, whereas short-term exposure to high amounts will cause high liver levels. After exposure has ceased there will be a redistribution so that eventually the renal levels will increase and liver levels decrease. The accumulation of cadmium in these two organs is related to the metabolism of metallothionein, the cadmium-binding protein. If renal damage occurs due to the accumulation of cadmium, the excretion of cadmium will increase, which may decrease renal levels considerably.

In the experiment by Yoshikawa, 1973 (section 4.2.1), it was seen that both at 6 and 24 hours after the last injection the liver concentrations of cadmium were significantly higher, more than 50%, in the pretreated group than in the other group. In other organs there was no such statistical difference between the groups. The author concluded that pretreatment accelerated the accumulation of cadmium in the liver.

Matsubara-Khan, 1974, studied the fate of radioactive cadmium, ^{109}Cd as a subcutaneous injection, the dose being 1.7 μCi/mouse. Animals were killed at different intervals from 1-128 days. Matsubara-Khan applied mathematical models for calculating biological half-time from the empirical data. When the average cadmium concentration in a given organ decreased among 10 mice already from the first day after injection a one-compartment model was used; when organ concentrations showed a transitory increase at the beginning a two-compartment model was used. Maximum liver levels were reached on day 8 after injection. Thereafter there was a decline in liver levels to day 128. The biological half-time in liver was calculated to be about 63 days. Maximum renal levels of cadmium were reached about 32 days after injection, subsequent to which they slowly declined. The author calculated the biological half-time in kidney to be 990 days, but as seen in Figure 4:7 an obvious decrease took place from day 32 to 128, which may indicate that the half-time in mouse kidney in fact is shorter than calculated. She also studied the salivary glands and estimated the half-time there to be 433 days. When the same author followed the fate of orally administered ^{109}Cd, 5 μCi/mouse, half-times in liver and kidney were 43 and 433 days respectively.

Ogawa, Suzuki and Tsuzuki, 1972, studied the fate of ^{115m}Cd after intraperitoneal injection to mice, the dose being 0.5 μCi. Radioactivity in whole body and excrements was determined day after day for different time periods. In one distribution study 5 animals were sacrificed 1, 2, 6 and 24 hours after injection. The uptake was highest in the liver, Initially, the pancreas took up more cadmium than did the kidney, but after 24 hours kidney levels had exceeded the pancreatic levels.

The cadmium distribution after repeated exposure has been described by Levy et al., 1973. Three groups of rats with 25 animals in each were given weekly injections subcutaneously of 0.087, 0.044, 0.022 mg cadmium as cadmium sulfate for two years. 75 animals constituted a control group given only water injections. The animals were killed after 2 years and cadmium determined in organs. Due to losses of water during storage cadmium concentrations were expressed as µg Cd/g "stored tissue", which makes it impossible to compare this with other studies. However, in all groups the kidney had the highest content of cadmium, followed by the liver except in the highest exposure group, where the spleen had a higher concentration of cadmium than the liver.

The accumulation in rabbits ingesting cadmium has been reported by Nomiyama, 1974. Food containing 300 µg/g of cadmium was given to a group of rabbits for 1 year. As shown in Figure 4:8 maximum levels in renal cortex were reached after about 20 weeks, and after 30 weeks there was a decline. In the liver maximum levels were reached at about 40 weeks of exposure, this organ also showing a decrease later. Nomiyama reported that these animals had signs of renal dysfunction at a concentration of about 200 µg/g in renal cortex, i.e. after about 15 weeks (section 5.1.1.2). The decrease in renal concentrations of cadmium was explained by Nomiyama as being the result of a decreased ingestion of cadmium as shown in Figure 4:9. The data will be commented upon further in section 4.2.3.1. The decreased renal cadmium concentrations occurred subsequently to an increased excretion after renal damage.

Suzuki, 1974, gave rats 0.5 mg/kg body weight subcutaneously each day except Sunday during 15 weeks. Each week 3 - 5 animals were killed and cadmium concentration was analyzed in organs. Blood and urine samples were collected weekly. Cadmium was analyzed with atomic absorption directly on the acid solution after wet ashing. The method will involve errors in analysis of low-level samples but at the concentrations received in this animal experiment the method may be used. Cadmium concentration in kidney increases until the 7th week and then levels off at about 150 µg/g wet weight (Figure 4:10). Liver concentration increases a little more and then decreases. In the water-soluble fraction for both kidney and liver about 80% of cadmium is found. Cadmium concentration in blood increases during the whole experiment (Figure 4:11). Nevertheless there is a great increase in urinary cadmium excretion after about 8 weeks (Figure 4:11). Urine volume and proteinuria had started an increase somewhat earlier. The data by Suzuki, 1974, speak in favor of an increased urinary cadmium excretion coinciding with the occurrence of renal damage and with the levelling off of cadmium concentration in kidney. In this experiment kidney damage occurred at a kidney cortex concentration of about 225 μg/g wet weight (= 1.5 x 150 μg/g).

The distribution of cadmium in lambs given cadmium orally for 191 days has been reported by Doyle et al., 1974. Five groups of six lambs in each were given diets containing 0, 15, 30 and 60 μg/g. In liver and kidneys cadmium levels were proportional to the dose. In kidney cadmium concentrations were on an average 768.8 μg/g dry weight (about 150 μg/g wet weight) in the group given the highest dose.

Ninety-day studies were performed on rats and dogs by Lorke and Löser, 1974. Groups of 20 male and female rats were given 0, 1, 3, 10 and 30 μg cadmium (as the chloride) per gram of diet. Cadmium was determined in liver and kidneys, the highest dose level resulting in mean levels of cadmium of about 9 and 12 μg/g wet weight respectively. There was no difference between the sexes. In the dog study, 2 female and 2 male dogs in each group, the same concentrations in food were used. Mean levels in liver and kidneys in the highest exposure groups were about 6 and 16 μg/g wet weight respectively. Cadmium was also determined in pancreas and salivary glands, being about 0.6 and 0.4 μg in the highest exposure group. Of interest is that at a lower exposure (1-3 μg/g diet) the salivary gland contained more than the pancreas.

Cousins, Barber and Trout, 1973, gave growing swine cadmium in the diet, the levels being 0, 50, 150, 450, and 1350 μg/g for 6 weeks. In another experiment 30, 90, 270, and 810 μg/g of diet were given. In liver the cadmium concentrations increased in proportion to exposure. Renal concentrations increased to about 275 μg/g wet weight, and then levelled off as shown in Figure 4:12. Since renal function and protein excretion were not examined, it is not known whether tubular dysfunction was present.

Tanabe (cited by Ishizaki, 1972) followed the fate of cadmium after cessation of long-term oral exposure. Adult rats were given cadmium as the chloride in drinking water (50 µg/g) for 6 months, whereafter the animals were studied for another 9 months. Every third month during and after exposure 2-4 animals of each sex were killed and cadmium determined in organs (method not stated). Liver levels of cadmium both after 3 and 6 months of exposure were only about 3 µg/g wet weight and at the end of the experiment about 2 µg/g wet weight. In kidney the levels were about 5 and 8 µg/g wet weight after 3 and 6 months respectively, about 10 µg/g the first months after cessation of exposure and at the end of the experiment a slight decrease was noted. It is clearly stated in the paper that the concentration in water was 50 µg/g but the concentrations in organs are surprisingly low, since several other experiments described in earlier editions of CITE and in the present one show considerably higher organ concentrations at this level of exposure.

4.2.3 Excretion

In CITE, 2nd ed., it was concluded that urinary excretion of cadmium was mainly dependent on body burden, before renal damage had occurred, but that considerably higher excretion could be caused by renal dysfunction or by extreme exposures.

4.2.3.1 Via urine

Results from several studies on urinary excretion of cadmium have been reported by Nomiyama, 1974. In Figure 4:13 the urinary excretion of cadmium in rabbits given 300 µg Cd/kg (36 mg of cadmium per day) of diet for one year is shown. Signs of renal dysfunction in the form of aminoaciduria were reported after about 16 weeks of exposure (corresponding to about 200 µg/g in renal cortex) and in the form of proteinuria after about 38 weeks (corresponding to about 300 µg/g in renal cortex). However, Nomiyama et al., in press, revealed that aminoaciduria occurred in 5 out of 16 determinations between 14 and 52 weeks of exposure and proteinuria occurred in 5 out of 7 determinations between 34 and 52 weeks of exposure. Nomiyama, 1974, concluded from these data that there is no increase in excretion of cadmium when renal dysfunction occurs. If Nomiyama's data are taken at face value the urinary excretion (µg Cd/day) does not increase during the first 25 weeks after which the volumes on an average are higher. This increase in excretion coincides with a decrease in liver and kidney values. However, it is not clear how urine was separated from feces and the risk for contamination from feces to urine must have been great (0.1% of fecal cadmium in urine would give about 35 μg/day). Contamination from food can also occur in such experiments. Excretion values of about 20 μg/day after only about 5 weeks of exposure were found at a time when the body burden was still relatively low, indicating that contamination could have taken place.

In the paper by Nomiyama, 1974, an experiment is reported in which rabbits were given subcutaneous injections of cadmium, 1.5 mg/kg, daily for 5 weeks. In Figure 4:14 the urinary excretion is shown. It is obvious that there is a considerable increase in cadmium excretion in the fourth week of exposure as also seen in the experiment by Suzuki, 1974, described in section 4.2.2 and Figure 4:11. Nomiyama also followed the excretion of cadmium for 15 weeks after cessation of exposure (daily subcutaneous injections of 1.5 mg Cd/kg for 3 weeks). Some animals were given repeated injections of uranylacetate after cessation of exposure which maintained a renal damage. These animals did not differ from the other animals with regard to decrease in urinary cadmium, as shown in Figure 4:15. There were lower cadmium levels in the kidneys of uranium treated animals.

Ogawa, Suzuki and Tsuzuki, 1972, gave a single dose of $^{115m}CdCl_2$ 80.5 μCi (specific activity not stated) to mice intraperitoneally. In Figure 4:16 it is shown that urinary excretion after an injected dose is low compared to fecal excretion. This is in accord with earlier studies. The influence of pretreatment with a small dose of cadmium upon urinary excretion after a larger dose was studied by Yoshikawa, 1973. Two groups of four male rats each received an intraperitoneal dose of 3 mg Cd/kg as the chloride, one group having received 0.6 mg Cd/kg intraperitoneally 24 hours earlier. Cadmium in urine was determined daily for one week by atomic absorption (no details given), and since the daily excretion was as high as 1.5 μg before injection, there is some doubt about the validity of the method. On the second day after injection the pretreated group excreted on an average about 4 μg of cadmium, whereas in the non-treated group about 20 μg was excreted. It was concluded that the excretion of cadmium was suppressed by pretreatment.

4.2.3.2 Via bile, pancreas, and gastrointestinal tract

Cikrt and Tichy, 1974, studied the excretion of cadmium via bile. Three groups of female rats with cannulated bile ducts were given intravenous injections of 67, 90, and 120 μg cadmium as cadmium chloride respectively (about 0.35, 0.45 and 0.60 mg/kg of body weight). Bile was collected hourly for 24 hours. In the three groups 0.83, 1.18 and 5.68% of the given dose was recovered in the bile during 24 hours, the main part being excreted during the first two hours after injection. Analysis of gastrointestinal content and feces showed that both in rats given 67 and 120 μg about 5.5% of the given dose had been excreted. In the walls of the gastrointestinal tract 7.04 and 4.79% of the dose was found. In another experiment on rats the excretion via bile was followed with 5-minute collections for 80 minutes after the injection of 10 μCi of ^{115m}Cd (95 μg) as the chloride. It was found that maximum excretion occurred between 15 and 30 minutes after injection. Electrophoretic separation of the radioactive bile in polyacrylamide gel revealed that cadmium was in two major fractions.

Further studies by Havrdová, Cikrt and Tichý, 1974, showed that when bile from rats given intravenous injections of cadmium chloride 120 μg Cd/rat, 0.6 mg/kg, was separated by gel filtration on Sephadex G-100 about half of the cadmium was in a high molecular weight fraction and the other half in a low molecular weight fraction. These fractions have not yet been characterized.

Nordberg and Robèrt, to be published, gave subcutaneous injection of ^{109}CdCl$_2$ (0.5 mg Cd/kg) to 4 rats. During the first hours after injection the biliary excretion per hour was about 0.04% of the given dose and after 68 hours a total of 0.29% of the given dose had been excreted. Separations by gel filtration showed that all cadmium was in fractions of very low molecular weight (< 4000). The differences in injection routes may explain the differences between the results of Nordberg and Robèrt, and Havrdová, Cikrt and Tichý. Nordberg and Robèrt also studied pancreatic excretion of cadmium in 4 rats. After 24 hours 0.0044% of the dose on an average had been excreted via that route.

4.2.4 Biological half-time

In CITE, 2nd ed., it was concluded that after a single injection of cadmium the biological half-time was about 200 days in mice and rats, 400 days in dogs and 1.5 years in squirrel monkeys.

Moore et al., 1973, determined the biological half-time in the rat after inhalation (section 4.1.1), ingestion and two injection routes. The half-time was found to be about 200 days by all routes, which agrees well with earlier data.

Sayato,Hasegawa and Ando, 1971, estimated the half-time in rats to be 15.3 days after an oral dose of ^{115m}Cd, but this was only based on observations during days 3 - 24 after exposure. They also gave an intravenous injection of ^{115m}Cd and estimated that the half-time was 333.7 days after 70 days of observation.

Matsubara-Khan, 1974, gave 40 male and 40 female mice subcutaneous injections of $^{109}Cd\ Cl_2$ and determined activity in organs in 5 males and 5 females at different intervals for 128 days (section 4.2.2). The following half-times were obtained: liver: 63 days, kidney:990 days, salivary glands: 433 days. Based on organ measurements the biological half-time for whole body was estimated to be 217 days. She also made a study on the fate of cadmium after oral administration and found the following half-times: liver: 43 days, kidney 433 days, salivary glands 150 days.

With regard to the injection experiment it has already been commented (section 4.2.2) that the estimate for half-time in kidney does not fit with the actual data as shown in Figure 4:7 where a clear decrease is seen in kidney from day 32 to 128. This seems to indicate a half-time of 300-400 days, which fits with the results from the oral experiment.

4.2.5 Interactions between cadmium and other metals or compounds

In CITE, 2nd ed., it was concluded that cadmium metabolism was intimately related to zinc metabolism. Another metal that had been shown to interact with cadmium was selenium. Chelating agents had been found to influence renal levels of cadmium after a single injection of cadmium. BAL increased the uptake, whereas HEDTA and DTPA decreased the uptake of cadmium in kidneys. It had also been shown that when a mixture of EDTA and cadmium was injected into rabbits, urinary cadmium excretion increased 1,000-fold, and organ levels of cadmium were lower than in animals given cadmium alone. Long-term oral exposure to NTA and cadmium revealed a tendency for NTA to cause less retention of cadmium.

With regard to zinc further data have been furnished on the relationship between cadmium and zinc in kidneys. Piscator, 1974, found an equimolar increase of zinc and cadmium in normal horses in the same range as earlier found in human beings, i.e. up to about 75 μg/g wet weight in renal cortex. At higher cadmium levels, however, the increase in zinc did not keep up with the increase in cadmium, as seen in Figure 4:17, where also results from some other studies are depicted. These findings indicate that in the range of 100-200 μg cadmium/g wet weight in renal cortex, i.e. below the critical level, an unfavorable quotient between zinc and cadmium exists, which may influence e.g. some enzymatic activities.

Cousins, Barber and Trout, 1973, gave growing swine cadmium in the diet, 0, 50, 150, 450 and 1350 μg/g of diet. Cadmium and zinc were determined in whole kidney and as seen in Figure 4:17 the increase in zinc was equimolar to the increase in cadmium at lower exposure levels up to a cadmium concentration of 78 μg/g wet weight (about 100 μg/g wet weight in renal cortex). Further increase in cadmium did not cause any further increase in zinc levels. In other organs there was a tendency for lowering of the zinc concentrations, most noticeable in the lung.

Evans, Grace and Hahn, 1974, studied the influence of copper and cadmium on the metabolism of ^{65}Zn in zinc-deficient and zinc-supplemented rats. Seventy-two male rats were fed a zinc-deficient diet for 13 days. One-half of the animals were zinc-supplemented by injections for two days. Oral doses of ^{65}Zn at three dose levels, 0.065, 0.65 and 6.5 µg were given by gastric tube to all animals. In each group one third was given copper and one third was given cadmium (112 µg of Cd as $CdCl_2$), e.g. the molar ratios of Cd to Zn were 1 000, 100 and 10 respectively. ^{65}Zn activity was measured in the carcass, excluding the gastrointestinal tract, and in the small intestine.

In zinc-deficient animals cadmium had no effect on the absorption of zinc; in zinc-supplemented animals, however, cadmium decreased the absorption of zinc at all dose levels of zinc with about 50 %. The uptake of zinc into the intestinal mucosa was decreased by cadmium in both zinc-deficient and zinc-supplemented rats, the decrease being about four-fold.

The results were interpreted as showing two independent processes for zinc absorption, one being the transport of zinc from the intestinal lumen to the carcass, the other being the uptake of zinc by the intestinal epithelial cells. The authors could not explain why cadmium (and also copper) did not influence the absorption into the carcass in zinc-deficient animals, but inhibited transport in zinc-supplemented ones. They postulated that the number of available zinc-binding sites in plasma albumin might be greater in zinc-deficient animals.

Shank and Vetter, 1974, gave groups of rats intraperitoneally 25 µg Cd/kg of body weight ($^{115m}CdCl_2$). Subgroups of 5 were simultaneously given copper, mercury or zinc at three different levels. The zinc to cadmium ratios were 50, 100 and 200 respectively (weight by weight). The animals were killed 24 hours after injection. Zinc was found to increase cadmium levels in liver as seen in Figure 4:18 (similar results were obtained with copper and mercury). It is seen that the uptake of cadmium in liver is increased about two-fold by the two highest zinc doses. The authors concluded that the studied metals produced an increase in liver levels of cadmium, suggested as being due to a stimulation of metallothionein synthesis.

Ogawa, Suzuki and Tsuzuki, 1972, found that the simultaneous injection of $ZnSO_4$ did not cause any change in the distribution of cadmium in mice given an intraperitoneal injection of ^{115m}Cd. They also found that mercury caused a substantial decrease in renal levels of cadmium, but a slight increase in liver levels.

Roberts et al., 1973, gave 11 male calves a diet with 8.5 μg/g of zinc and some of these calves were also given 350 μg Cd/g of diet for 14 days. After 8 days an oral dose of ^{65}Zn was given to all animals and feces and urine collected for 7 days, whereafter the animals were killed. This high exposure to cadmium decreased the retention of ^{65}Zn, as shown both by excretion measurements and tissue analysis. Stable zinc content in tissues was also determined. Zinc concentrations in liver and kidneys were higher in cadmium treated animals, the difference not being statistically different, whereas there was a significant decrease in muscle zinc.

These data support the above mentioned findings by Evans, Grace and Hahn, 1974, that cadmium will decrease the absorption of zinc in zinc-supplemented animals and also confirm earlier findings that cadmium exposure causes a redistribution of body zinc.

The influence of mercury was studied by Suzuki and Yoshikawa, 1971, who gave intraperitoneal injections of 3 mg/kg body weight of cadmium chloride to groups of rats without and with mercury pretreatment in a dose of 0.3 mg/kg, 24 hours before the cadmium injection. Animals were killed 96 hours after the cadmium injection. It was found that the cadmium content of the liver was less in animals pretreated with mercury. This result is in contrast to the results by Ogawa, Suzuki and Tsuzuki, 1972, but the mercury dose was considerably less.

It has earlier been shown that ascorbic acid influences the toxicity of cadmium. Pyridoxine (vitamin B_6) was studied by Stowe et al., 1974. Groups of rats were given diets deficient and high in pyridoxine (two levels) and with and without added cadmium (100 µg/g diet) for 12 weeks. Pyridoxine supplemented rats had higher concentrations of cadmium in kidney and liver than pyridoxine-deficient animals, and it was also found that increasing the pyridoxine intake increased the retention of cadmium. Since pyridoxine also increased the toxic effects of cadmium the authors concluded that excessive intakes of pyridoxine might aggravate the effects of cadmium.

Eybl, Sykora and Mertl, 1973, gave mice a single injection intravenously of cadmium chloride (0.6 mg ^{115}Cd/kg). Some animals were also given simultaneously an intraperitoneal injection of zinc chloride (8.9 mg Zn/kg). After 24 hours the animals were killed. There was a tendency to higher Cd-retention in animals given zinc, but the difference was not statistically significant. In liver 40.1% of the injected dose was found in the animals given Cd alone, whereas in zinc-treated animals 35.9% was found. In kidneys the corresponding figures were 3.3 and 4.0% respectively. Only in testicles did zinc cause a significant increase in cadmium concentrations. In another experiment mice were given an intravenous injection of 0.25 mg ^{115}Cd/kg as the chloride, and followed for 21 days. On day 7, 10, 13, 17 and 20 after the cadmium injection, intraperitoneal doses of 8.1 mg Zn/kg were given. After 21 days the animals were killed. The whole body retention of cadmium was slightly higher in animals that had received zinc. Liver retention was slightly lower in zinc-treated animals, 51.8% compared to 55.9%, and kidney retention was higher, 9.5% compared to 5.7%.

These results indicate some influence of zinc on cadmium metabolism in injection experiments. It should be noted that these results are in contrast to those of Shank and Vetter, 1974.

Eybl, Sykora and Mertl in the same study also tested different chelating agents under the same experimental conditions. In the acute experiments CaDTPA and ZnDTPA were found to be the most effective substances for removal of cadmium. After 24 hours the retention of cadmium was only about 20 and 30% respectively, of the injected dose, whereas about 80% remained in animals given only $CdCl_2$.

In the 21-day experiment CaDTPA, ZnEDTA and ZnDTPA caused very small but significant decreases in whole body retention compared to day 7, as seen in Figure 4:19. The differences are much smaller than in the acute experiment. In organs the differences were small, the liver retention being in the range of 50.8-57.5% in the animals given chelating agents compared to 55.9% in animals given only Cd; in kidneys the range was 5.3-6.0% compared to 5.7%.

Histological examinations of the kidneys were not made. These studies confirm that chelating agents may have a dramatic effect on excretion and distribution of cadmium if given simultaneously, but that these agents have very little influence on the distribution of cadmium if treatment is begun several days after cadmium exposure.

In another experiment 112 mg/kg body weight of cadmium as the chloride was injected subcutaneously into rats. Simultaneously, the Ca- and Zn-complexes of chelating agents were injected intraperitoneally. Urine was collected and after 2 hours the animals were killed. In urine cadmium complexes were found and characterized.

Dequidt, Haguenoer and Fromont, 1973, gave rats single doses of cadmium, 2 mg/kg as the sulfate, by injection and then for 7 days different agents, penicillin (30,000 I.U.), BAL (3 mg/kg), CaN_2EDTA (25 mg/animal), penicillamine (50 mg/animal) and $CaNa_3DTPA$ (10 mg/animal).

Penicillin did not cause an increase in urinary excretion of cadmium, but liver concentrations were only about one fourth of control levels (21 and 78 μg/g respectively). In other organs examined, there were no major differences. Intestinal concentrations were not higher in the treated animals and it is not clear where cadmium was excreted or stored.

The other drugs examined behaved as expected, i.e. urinary excretion increased to some extent and liver and renal levels decreased. The authors concluded that none of these drugs would be suitable for treatment of cadmium poisoning.

In studies on selenium toxicity Hill, 1974, found that dietary levels of 57 μg Cd/g caused a 15% mortality and a level of 40 μg Se/g of diet caused a mortality of 20% in chicks. When cadmium and selenium were given together at the above concentrations mortality was 21.2%. In controls with no extra selenium or cadmium, mortality was 2%. Selenium caused growth retardation and so did cadmium, but together they did not cause more growth retardation than selenium alone. The only conclusion that can be drawn from these results is that there is no additive effect.

4.2.6 Conclusions

A vast number of experiments have been performed during the last years, but the majority have mainly just confirmed earlier findings. Among new data are those showing that biliary excretion of cadmium in animals is low. The urinary excretion of cadmium is still a matter of discussion, but no conclusive evidence has been presented that would speak against an increased excretion concurrent to tubular dysfunction. The difficulties in performing long-term feeding experiments with high doses of cadmium without getting contamination of urine from feces are obvious. Furthermore there are other studies that show a sharp rise in urinary excretion of cadmium when damage occurs.

Studies on interaction between cadmium and zinc and other metals stress the importance of further research in that field.

4.3 TRANSPORT, DISTRIBUTION AND EXCRETION OF CADMIUM IN "NORMAL" AND EXPOSED HUMAN BEINGS

4.3.1 Transport and distribution in blood

In CITE, 2nd ed., it was concluded that the average normal cadmium level in whole blood is well below 10 ng/g (1 μg/100 g). In exposed workers considerably higher values had been reported. After exposure had ceased, there would be a slow decrease in blood concentrations. This decrease could, however, be relatively fast after short-term intensive exposure.

During the last years a large number of studies have been performed on "normal" blood concentrations. The results of these studies are compiled in Table 4:1. Most studies give mean concentrations between 1 and 10 ng/g. The lowest values are reported by Ediger and Coleman, 1973,and Ulander and Axelsson, 1974, both groups using the Delves' Cup technique. It is noteworthy that in some studies a separation has been made into smokers and non-smokers, the former generally having higher blood concentrations than the latter.

In all studies atomic absorption has been used with different procedures or equipment, see also Chapter 2.

Even though the present data strongly support the earlier finding that "normal" blood levels of cadmium are below 10 ng/g, the "true" levels are not known yet.

In some of these reports results from cadmium determination in blood from exposed workers have been included (Cernik and Sayers, 1974; Lauwerys, Buchet and Roels, 1973; Roels et al., 1974; Lauwerys et al., 1974).Mean values between 10 and 50 ng/g have been reported for different exposure groups, exposure to cadmium fumes giving the highest values.

That the decrease in blood concentrations of cadmium after long-term low level exposure is very slow is shown in Figure 4:20 illustrating blood levels in a worker during exposure and for 4 years after cessation of exposure (Rogenfelt, personal communication). After high exposure there is a much more rapid decrease as seen in Figure 4:21. Initially extremely high blood cadmium concentrations had been recorded.

4.3.2 In organs

Data on concentrations of cadmium in organs have been presented in CITE, 2nd ed. Average liver concentrations of cadmium were between 1-3 μg/g wet weight in some European countries and in the U.S., whereas "normal" concentrations in three Japanese areas were considerably higher, in one area 5-10 times higher than in the U.S., U.K. and Sweden. In exposed workers and Itai-itai disease patients cadmium concentrations in liver could reach 100 μg/g wet weight or even higher.

The highest cadmium concentration was found in the renal cortex, the "normal" concentration being on an average between 15 and 30 μg/g wet weight at age 50 in some European and American areas. Smokers generally had higher cadmium levels than non-smokers.

In three areas in Japan, mean normal levels of 50, 90 and 125 μg/g respectively were found at about 50 years of age. In exposed workers concentrations of about 300 μg/g wet weight had been reported, but also values within the normal range had been found despite signs of cadmium intoxication. Losses of cadmium due to renal dysfunction were thought to be the cause of these low values.

It was calculated that normally about 50% of the total body burden was stored in liver and kidneys, the kidneys alone containing about one third. At high exposure the liver contained a larger proportion of the body burden.

Hamilton, Minski and Cleary, 1972/1973, analyzed cadmium in 8 kidneys from accidental deaths in U.K. by X-ray fluorescence. The mean concentration in renal cortex was 14.3 μg/g wet weight, but no data on age are given.

Plantin, 1973, and personal communication, determined cadmium by neutron activation in liver and renal cortex from 8 cases of sudden death (age 19-67 years) and in 8 subjects who died from coronary heart disease (age 55-63, mean age 58 years) in Uppsala, Sweden. If the latter group is compared with 4 cases (age 54-67 years, mean age 58) from the first group, the levels in liver were 1.8 and 1.0 μg/g wet weight respectively, and in renal cortex 20.1 and 13.3 μg/g wet weight respectively. The values agree with other data on renal and liver levels in Sweden and Europe in persons of similar age, but since smoking habits are not known it is difficult to evaluate the differences in cadmium levels between the groups.

Vuori, Huunan-Seppälä and Kilpiö, 1974, determined cadmium in whole kidney from 20 accident cases (age 13-78 years, mean 35) in Finland. The average concentration was 73.2 μg/g (S.D. 38.2) dry weight, which would correspond to about 22 μg/g wet weight in renal cortex. The mean concentration in liver was 4.15 μg/g dry weight (about 1.3 μg/g wet weight).

In California, U.S.A., Indraprasit, Alexander and Gonick, 1974, analyzed for several metals, among them cadmium, in 220 random hospital autopsies. The patient population was divided into three groups, the first one comprising 116 cases called "normal" with regard to renal function (serum creatinine having been 1.5 mg/100 ml). The other two groups were composed of 52 cases of acute renal failure and 52 cases of chronic renal failure.

Cadmium was analyzed by emission spectrography. In Figure 4:22 cadmium concentrations in renal cortex in the "normal" group are shown in relation to age. The highest concentrations are at age 31-60, being on an average 107 μg/g dry weight (about 21 μg/g wet weight). The "normal" group was further divided into normotensives and hypertensives; the average cadmium concentrations were the same in both groups. Liver levels of cadmium were on an average 14.4 μg/g dry weight in the "normal" group (about 4.4 μg/g wet weight). The "normals" in age 31-60 were also compared to the groups of the same age with renal failure, the latter having significantly lower cadmium and zinc concentrations.

In Denmark Östergaard and Clausen, 1974, determined cadmium in kidneys from 30 autopsied persons from 0-85 years of age. In age groups 40-49, 50-59 and 60-68 mean renal cortex levels were 38 (n = 4), 33 (n = 5) and 35 (n = 7) μg/g wet weight respectively. These concentrations are slightly higher than earlier reported from neighboring countries.

Hirst et al., 1973, compared 12 autopsy cases with a history of emphysema (age 48-83 years) with a matched control group (48-87 years). The mean renal levels of cadmium were 3,000 and 1,700 μg/g ash respectively. Corresponding levels for liver and lung were 230 and 63, and 83 and 19 μg/g ash respectively. Both groups were mainly composed of smokers, but in the emphysema group there were more heavy smokers. The authors concluded that smoking was the main cause of the differences in tissue levels of cadmium.

McKenzie, 1974, studied the cadmium, zinc and copper content in autopsies from New Zealand. 7 persons had died from hypertensive disease, 5 from neoplastic disease and the remaining 7 from other diseases. There were no statistically significant differences between cadmium concentration in liver, kidney, lung or pancreas depending on type of disease or smoking habits. The 6 persons in the 40-60 years age group had an average kidney (probably total) concentration of 220 μg Cd/g dry weight. According to the author the mean water content of the kidneys was 85% for all 19 cases. On a wet weight basis the cadmium concentration in the 6 persons will be 33 μg/g. The corresponding kidney cortex concentration would be about 50 μg Cd/g wet weight (x 1.5), which is slightly higher than in corresponding age groups in Europe and the U.S.

4.3.3 Excretion

4.3.3.1 Via urine

The normal urinary excretion of cadmium is low, around 2 μg/day or less. Under moderate exposure a relationship between body burden of cadmium and urinary excretion will be upheld. In exposed workers urinary excretion may vary within large limits, depending on actual exposure, body burden and renal function.

In Table 4:2 data are compiled from some recent studies on normal excretion of cadmium. In CITE, 2nd ed., several examples were given of studies where inaccurate methods were involved. Even though most reports now present results which seem to be relatively accurate there are still some deviating data. In these studies true account has not been taken of the interference which may occur in the determination of urine cadmium with atomic absorption.

Of special interest are the studies from Belgium, where the studied groups were separated into smokers and non-smokers. Smokers had higher urine cadmium than non-smokers, which agrees with the fact that smokers have higher body burdens.

Variations in daily urinary excretion of cadmium were studied by Taguchi et al., 1972. Five men collected urines for 5 days and cadmium was determined by atomic absorption after chelation and extraction into an organic solvent. In Table 4:3 mean values and variation coefficients are shown. In Figure 4:23 daily urinary excretion of cadmium is shown in relation to smoking habits.

Urine excretion in cadmium workers has been studied by several authors (Cernik and Sayers, 1974; Lauwerys, Buchet and Roels, 1973; Lauwerys et al., 1974; Roels et al., 1974; Piscator, unpublished data). Cernik and Sayers, 1974, reported on groups of workers exposed to cadmium oxide dust and fumes respectively, the latter excreting considerably larger quantities of cadmium. In the former group cadmium could not be detected in 8 out of 14 workers, in the remaining six concentrations from 3.9 to 47 μg/l were found. In 8 workers exposed to cadmium fumes the concentrations ranged from 9.7 to 75 μg/l, on an average 29 μg/l.

In Figure 4:21 the decrease of urine levels of cadmium after exposure has ceased is shown in 5 workers, who for a short period were exposed to large amounts of cadmium (Piscator, unpublished data). There were no signs of renal damage in 4 cases, whereas in the fifth there was a suspicion of a slight tubular dysfunction. The decrease in urine parallels the decrease in blood levels.

The new data confirm that the normal excretion of cadmium is low. Smoking has been shown to cause higher excretion of cadmium and since it also has been shown that smoking causes increases in body burden of cadmium, it is conceivable that the higher urine excretion reflects differences in body burden.

4.3.3.2 Via other routes

It was mentioned in earlier reports that cadmium could be found in human hair with concentrations generally below 1 μg/g and that hair analysis would be of little value for body burden estimates because of contamination risks.

Some studies on metal content of hair have appeared recently. Stanković et al., 1974, determined cadmium by atomic absorption in hair in 4 groups in Yugoslavia: 30 farmers, 40 city dwellers, 50 persons living in the vicinity of a lead smeltery and 50 workers in the same smeltery. Cadmium concentrations in hair were 0.22, 0.30, 0.33 and 0.78 μg/g respectively. In CITE, 2nd ed., a mean value of 0.54 µg/g was reported in 17 males in a rural area in Yugoslavia. In the study of Stanković et al. 1% nitric acid was used for washing the hair, which may have influenced the results.

Vuori, Huunan-Seppälä and Kilpiö, 1974, determined cadmium in hair from 20 lethal accident cases in Finland and found a mean concentration of 0.35 µg/g. In U.S.A. Petering, Yeager and Witherup, 1973, determined cadmium in hair from 95 men and 83 women in Cincinnati. In males there was an increase from about 1 µg/g at age 2-3 to about 2 µg/g at age 6, whereafter the level was about the same, until after age 20, when a decrease appeared. In females cadmium concentrations increased from about 1 µg/g at age 15 to about 2 µg/g at 30-40 years of age, whereafter there was a decrease.

A total of 652 hair samples from 200 persons were analyzed in a study in Houston, Texas (Johnson et al., 1974). There were three exposed groups, policemen, garage attendants, and women living near a freeway, each group having a control group. From each participant 3 or 4 hair samples generally were obtained. Mean hair cadmium concentrations in the six groups varied from 0.6-2.2 µg/g, the highest values being found in the control group for the garage attendants, and the lowest in the two female groups.

What is noteworthy is that in the recent papers just as in those reviewed in CITE, 2nd ed., there is a discrepancy between results obtained in the U.S. and in other areas. Mean values around 2 µg/g have repeatedly been found in adults in U.S.A., whereas in Europe mean values around 0.5 µg/g are generally found. Since there are no major differences in liver or renal levels of cadmium, and the analytical methods are similar, this discrepancy is surprising. In Japan lower hair concentrations have been found than in U.S.A. (Tsuchiya, Petering and Kobayashi, 1971).

4.3.4 Conclusions

Extensive studies on the occurrence of cadmium in blood have confirmed that the normal level is well below 10 ng/g. It has now also been possible to show differences between smokers and non-smokers of the same age, thus emphasizing the importance of cadmium inhaled from cigarettes.

Several new data have been obtained on cadmium concentrations in organs in "normal" populations. Data from U.S., U.K., Sweden, Denmark and Finland confirm that mean levels in adults may generally vary from about 15 to about 30 μg/g wet weight of renal cortex. In some studies the materials have been divided into smokers and non-smokers, the latter having considerably less cadmium in liver and kidneys.

With regard to daily urinary excretion of cadmium new data verify that "normal" excretion is around 1 μg/l or less in adult populations in Europe and U.S.A. Workers without direct exposure to cadmium, but living in the vicinity of cadmium emitting industries, had between 1 and 2 μg/l urine. Smokers were found to excrete more cadmium.

Earlier data on body burden and renal burden have been confirmed by the new data on organ concentrations. Since organ concentrations and urine concentrations are higher in smokers than non-smokers this gives further evidence that the urinary excretion is dependent on body burden.

There are no new data on biological half-time in human beings.

4.4 METALLOTHIONEIN: OCCURRENCE AND PROPERTIES

A review of previously published data on metallothionein has been given in CITE 2 nd ed., and will not be repeated here. During the last years the following additional data have been published:

dition to those of Bühler 1973, mentioned previously (CITE 2nd ed.). Bühler and Kägi, 1974, isolated two forms of metallothionein from human liver by means of precipitation and chromatographic procedures including final separation of the two forms by DEAE ion exchange chromatography. The two forms had a metal content of 6.53 (Zn: 6.26; Cd: 0.14; Cu: 0.13) and 7.84 (Zn: 7.58; Cd: 0.19; Cu: 0.07) percent (referring to the metal-free protein) and a similar amino acid composition with about 20 residues of cysteine and no aromatic ones in a minimum molecular weight of 6100.

The molecular weight was further studied on performic acid oxidized metallothionein by gel filtration in 6 M guanidinium HCl on Biogel A-5 m. This procedure gave a molecular weight of 6500 in relation to appropriate marker substances. These results are in accord with earlier reports by Nordberg et al. 1972, who found a similar molecular weight of metallothionein from rabbit liver.

Nordberg, Trojanowska and Nordberg, 1974, studied metal binding proteins from rabbit kidney. They found that the protein isolated from cadmium exposed animals had isoelectric properties similar to metallothionein previously isolated from rabbit liver (Nordberg et al., 1972). A mercury binding protein was also found which eluted on G-75 sephadex at the same place as metallothionein. However there was a markedly different isoelectric point of this protein. The authors also showed that mercury metallothionein prepared in vitro had different UV-spectrum than Cd/Zn containing metallothionein.

Studies on rat liver metallothionein relating partly to the mercury binding properties of the protein have been performed by Weser et al., 1972, 1973, and Rupp, Voelter and Weser, 1974, and particularly by Sokolowski, Pilz and Weser, 1974. These authors focused on the binding properties of the SH-groups in metallothionein.

By employing metallothionein with various metals bound to it (Zn^{2+}, Cd^{2+} or Hg^{2+}), and X-ray photoelectron spectroscopy they observed that the complete displacement of Zn^{2+} and or Cd^{2+} by Hg^{2+} results in a different energy of the binding electrons of the sulfur of the thiol group compared to the binding of zinc or cadmium.

Purification of rat liver and kidney metallothionein has been reported by Kimura et al., 1974. Their preparations, both from kidney and liver, had a high cysteine content, but contained small amounts of phenylalanine.

Equine hepatic and renal metallothionein has been further characterized by Kägi et al., 1974. These authors also found that their metallothionein had a molecular weight of 6500. These authors found a similar amino acid composition of the protein from both liver and kidney with a cysteine content of 19-20 residues per molecule. The metal composition was different in the two organs, zinc being the dominant metal in liver metallothionein and cadmium and zinc being present in almost equal molar amounts in the renal protein.

Isolation and identification of metallothionein from mice have been reported by Nordberg and Nordberg, 1973. Such a protein from the livers of mice has been further purified and characterized by Nordberg, M. (to be published). This protein too had a high cysteine content, no aromatic acids and a molecular weight of about 6000.

The role of metallothionein-like proteins in animal nutrition has been reviewed by Bremner and Davies, 1973. Bremner and Marshall, 1974, and Bremner and Davies, 1974, studied the distribution of copper and zinc among soluble proteins of livers from calves and sheep of different Cu and Zn status and from rats injected with Cu^{2+}. They found that the distribution of metals between these fractions was variable and dependent on both Cu and Zn status. In a following paper the authors further characterized the low molecular weight fractions from calf liver and showed that this protein, which had mainly zinc bound to it, had an amino acid composition similar to what has been reported for metallothionein.

Davies, Bremner, and Mills, 1973, and Bremner, Davies, and Mills, 1973, have reported that injection of zinc salt into rats induces the synthesis of a low molecular weight zinc binding protein in the liver and that food restriction, another means of increasing liver zinc concentration, also results in the appearance of this protein.

Studies on the appearance of a zinc-binding protein in rat pancreas have been performed by Davies and Bremner, 1974. This protein was found on gelchromatography to have a molecular weight similar to metallothionein.

The possibility of induction of metallothionein synthesis by various metal salts has further been studied by Sabbioni and Marafante, 1975, who could not find such induction by any metals other than cadmium.

Squibb and Cousins, 1974, have shown that metallothionein (cadmium binding protein) is synthesized in the liver de novo in response to injection of cadmium to rats. This reaction could be inhibited by known inhibitors of protein synthesis like actinomycin D.

TABLE 4:1

"NORMAL" LEVELS OF CADMIUM IN BLOOD µg/100 ml (OR 100 g) (METHOD: ATOMIC ABSORPTION)

Sex	Country	n	Age	mean, S.D.	range	special procedure	Reference
M (S)	Belgium	22	51·7	7.0±1.0		extraction	Lauwerys et al., 1974
M	"	110		5.8±2.1		"	Roels et al., 1974
M (S)	"	33	48·10	7.0±6.0		"	Lauwerys, Buchet and Roels, 1973
M (NS)	"	11	31·14	7.0±3.0		"	"
F (S)	"	14	30·7	12.0±15.0		"	"
F (NS)	"	15	28·9	8.0±18.0		"	"
M+F	Denmark	110	0-80	12.6	5.0-45.0	Graphite atomizer	Nygaard, Bonde and Hansen, 1974
M (S)	U.K.	9	Adults	23.2±5.7	15.0-28.5	Delves cup	Willden, 1973
M (NS)	"	10	"	20.6±4.4	11.0-28.0		
F (S)	"	8	"	24.4±1.9	21.0-27.0		
F (NS)	"	8	"	19.7±1.9	16.0-22.8		
M+F	West Germany	37	"	9.5	4.0-19.0		Stoeppler et al., 1974
M	USA	50	"	0.6	0.2-2.0	Delves Cup with backgr corr	Ediger and Coleman, 1973
M+F	"	60	12	0.5(median)			
	U.K.	10		4.0	2.0-8.0		
M (S)	Sweden	25	20-55	2.3	0.6-6.1	"	Ulander and Axelson, 1974
M (NS)	"	19	20-55	0.6	0.3-1.2	"	
F (NS)	"	20	20-55	2.0	0.5-7.6	"	
F (NS)	"	25	20-55	0.5	0.2-1.0	"	

TABLE 4:2

URINARY EXCRETION OF CADMIUM IN "NORMAL" SUBJECTS. METHODS: ATOMIC ABSORPTION

Sex	Country	n	age	mean, S.D.	range	unit	special procedure	Reference
M (S)^{xx}	Belgium	33	48±10	2.0±1.2		µg/g creatinine	extraction	Lauwerys, Buchet and Roels, 1973
M (NS)^{xx}	Belgium	11	31±14	1.0±0.7		µg/g creatinine	"	Lauwerys, Buchet and Roels, 1973
F (S)^{xx}	Belgium	14	30± 7	2.6±3.0		µg/g creatinine	"	Lauwerys, Buchet and Roels, 1973
F (NS)^{xx}	Belgium	15	28± 9	1.5±2.0		µg/g creatinine	"	Lauwerys, Buchet and Roels, 1973
M (NS)	Belgium	20		0.38±0.4				Lauwerys, Buchet and Roels, 1973
M^{xx}	Belgium	110		1.79±1.68			"	Roels et al. 1974
Not reported^{xx}	Japan	81	20-60	1.39	0.25-4	µg/l	"	Tada, Nakaaki and Fukabori, 1972
M	Japan	5	27-37	2.56^{x}	1.7-3.7^{x}	µg/day	"	Taguchi et al., 1972
M	Japan	374^{xxx}	20-60	$0.64^{+0.65}_{-0.33}$ (geometric mean)	0.05-3.68	µg/l (adjusted individually for spec. grav.)		Fukabori and Nakaaki, 1974
				$1.06^{+1.06}_{-0.53}$ (geometric mean)	0.24-6.72	µg/24 hr		Fukabori and Nakaaki, 1974
F	New Zealand	106	43± 8	23±21		µg/24 h	-	McKenzie and Kay, 1973
M+F	U.K.	10	Adults	44.8±12.4		µg/day		Willden, 1973
	U.S.A.	20	-	1.5±0.7	0.4-3.7	µg/l	graphite atomizer	Ross and Gonzales, 1974
	U.S.A.	13		2.9	0-10.1	µg/day	extraction Carbon Rod Atomizer	Kubasik and Volosin, 1973

^{x}Mean of 5 days

^{xx}Workers without direct exposure to metals

^{xxx}Including the 81 from Tada, Nakaaki and Fukabori, 1972.

TABLE 4:3

URINARY EXCRETION OF CADMIUM IN 5 SUBJECTS DURING 5 DAYS. (From Taguchi et al., 1972)

Case No.	Age	Smoking cigarettes/day	Cadmium µg/day	Coefficient of variation	Cadmium µg/l	Coefficient of variation
1	33	0	1.66±0.71	0.43	2.14	0.53
2	33	20	2.54±0.79	0.31	1.93	0.21
3	31	15	2.58±1.47	0.57	1.88	0.36
4	37	25	3.71±1.56	0.42	2.80	0.24
5	27	10	2.29±0.87	0.38	1.94	0.31

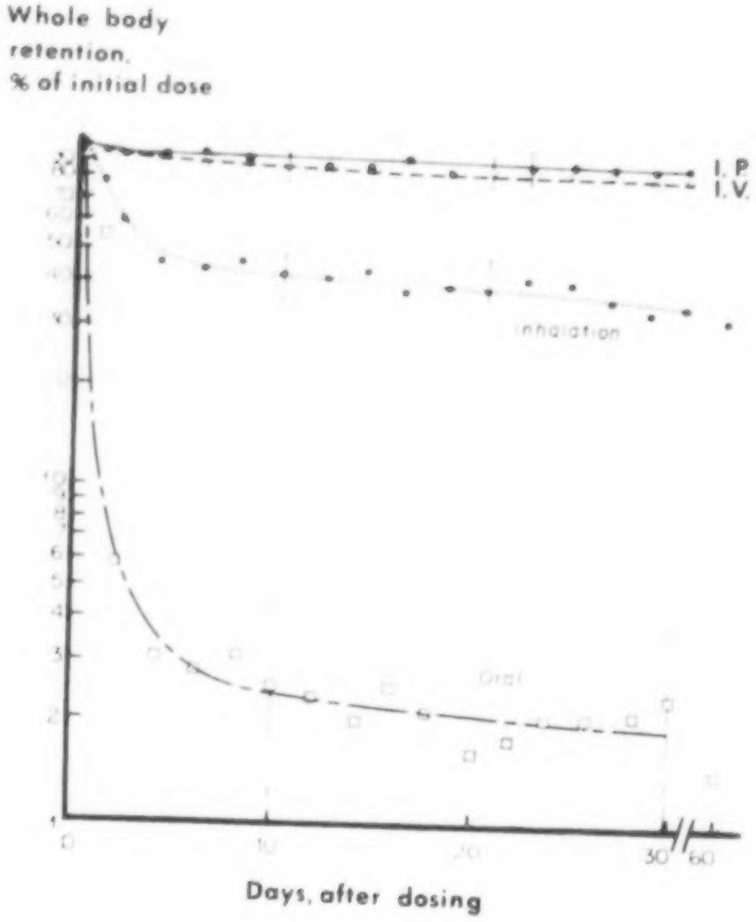

FIGURE 4:1 Whole-body retention of ^{115}Cd following a single exposure by different routes of administration (From Moore et al., 1973).

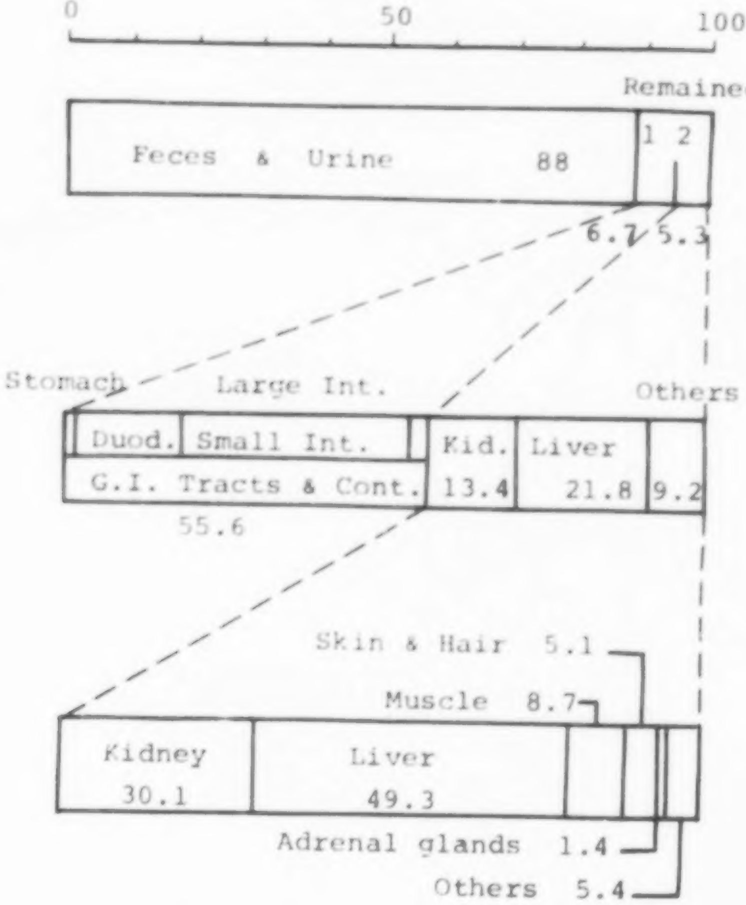

FIGURE 4:2 Absorption rate and organ distribution of cadmium after a single oral dose of ^{109}Cd to a monkey, Macaca irus. After 19 days (From Suzuki, Taguchi and Yokohashi, 1974).

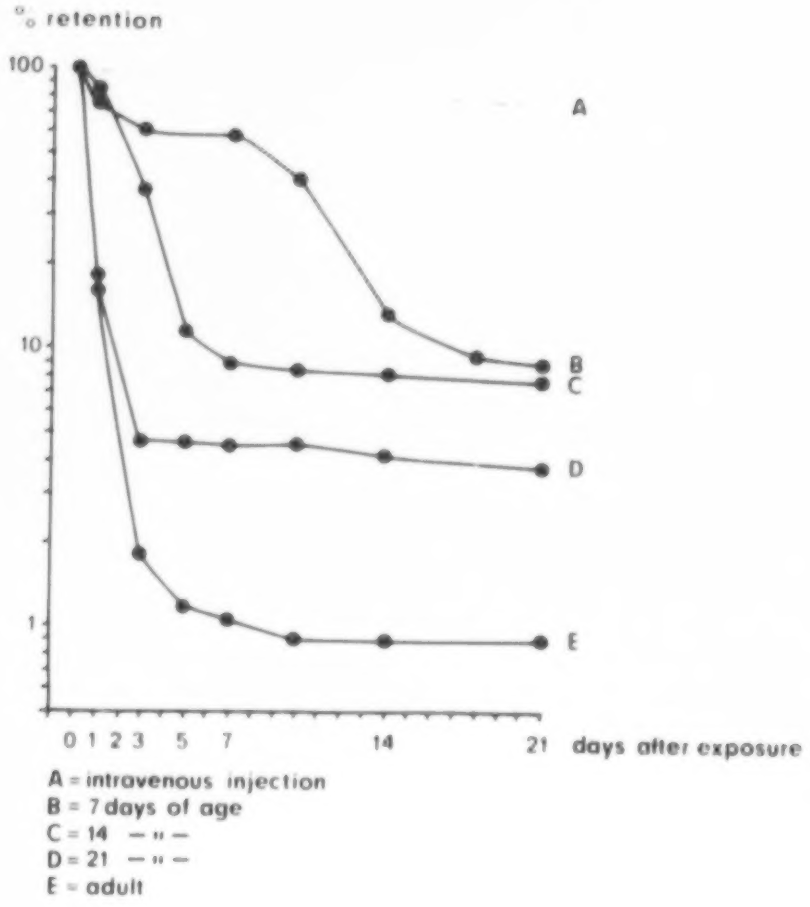

FIGURE 4:3 Whole-body retention after a single peroral exposure to ^{115m}Cd in mice of different ages (From Matsusaka et al., 1972).

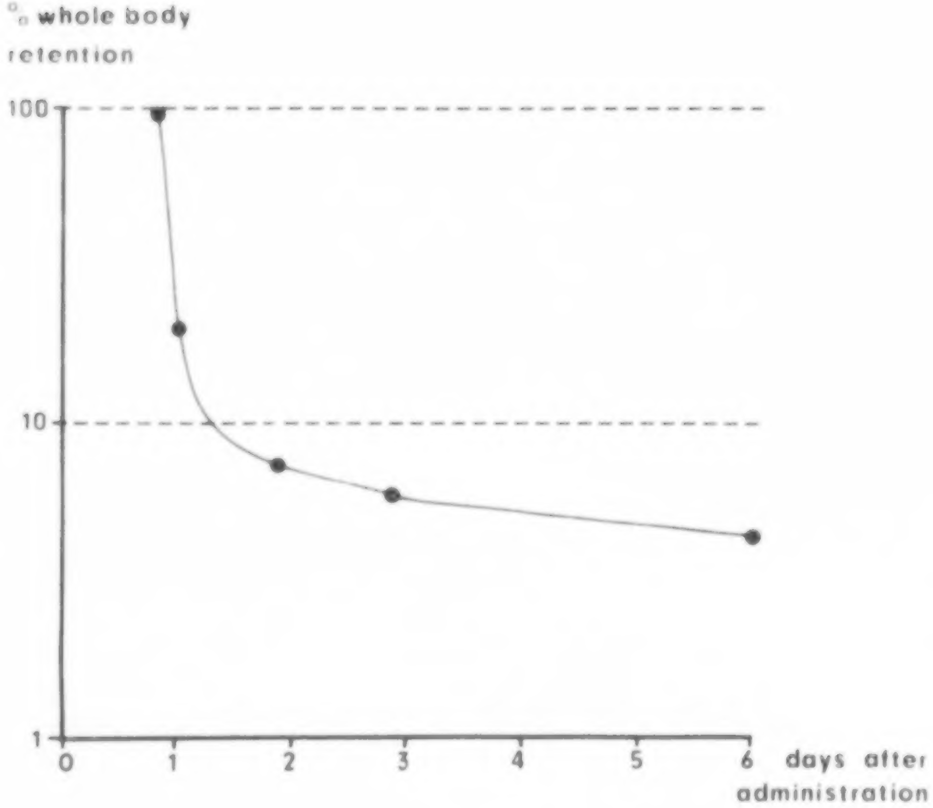

FIGURE 4:4 Retention of ^{115m}Cd in a man after oral administration (From Yamagata, Iwashima and Nagai, 1974).

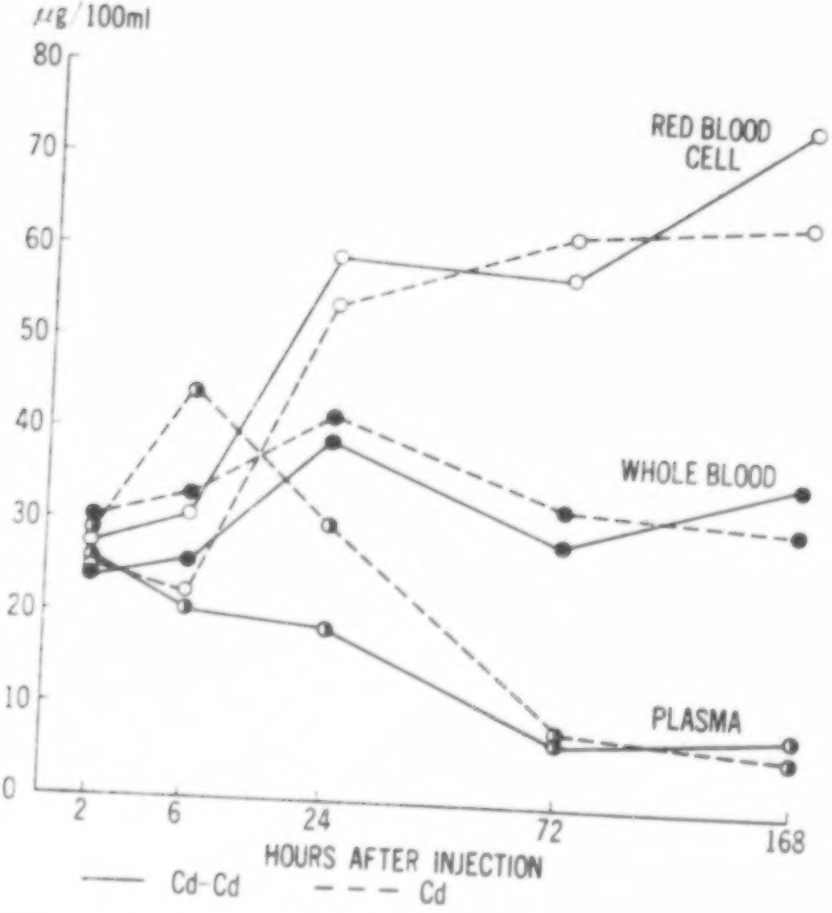

FIGURE 4:5 Changes of cadmium concentration in whole blood, red cells, and plasma after two exposure conditions in rats (continuous lines = pretreatment with 0.6 mg of cadmium/kg body weight prior to an i.p. injection of 3 mg Cd/kg. Dashed lines = rats given i.p. 3 mg cadmium/kg body weight, no pretreatment). (From Yoshikawa, 1973).

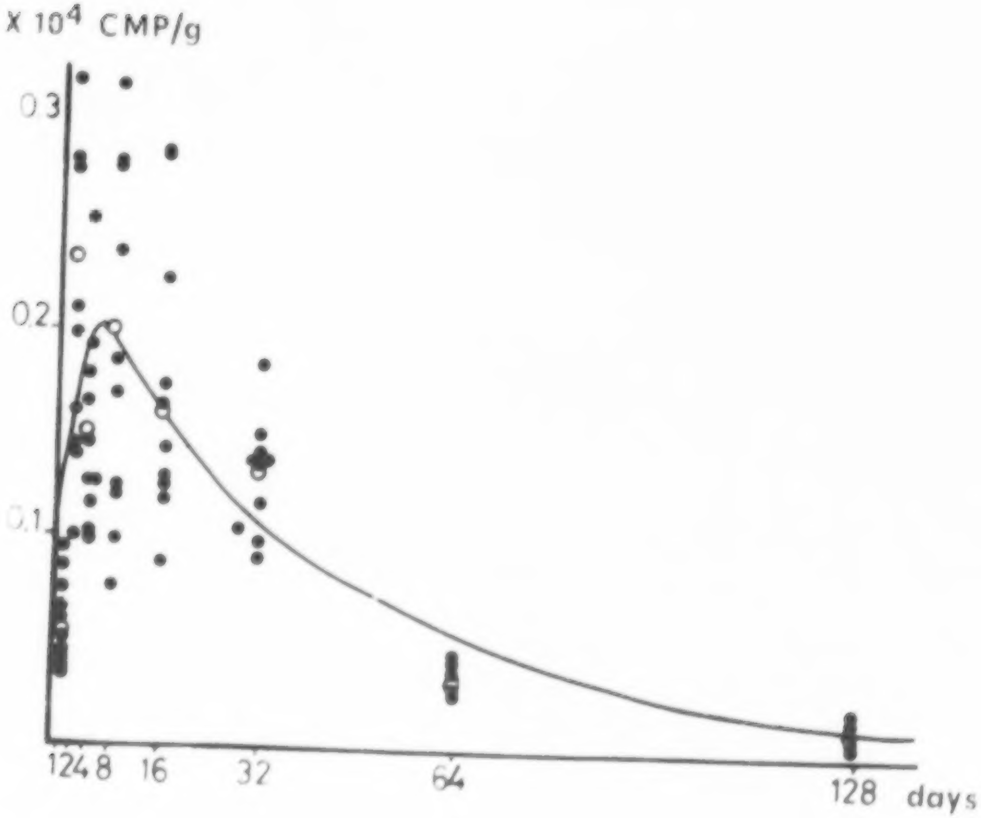

FIGURE 4:6 Blood levels of ^{109}Cd after a single subcutaneous injection (From Matsubara-Khan, 1974).

- • individual values
- average values

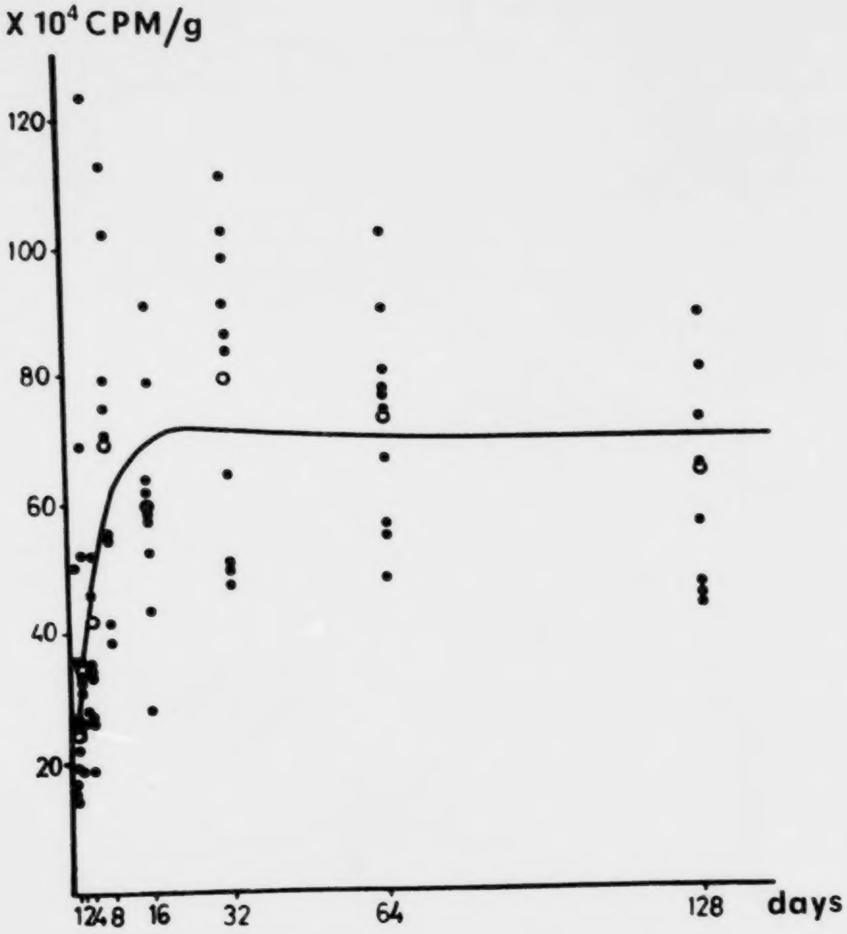

FIGURE 4:7 Renal levels of ^{109}Cd after a single subcutaneous injection (From Matsubara-Khan, 1974).

● individual values

○ average values

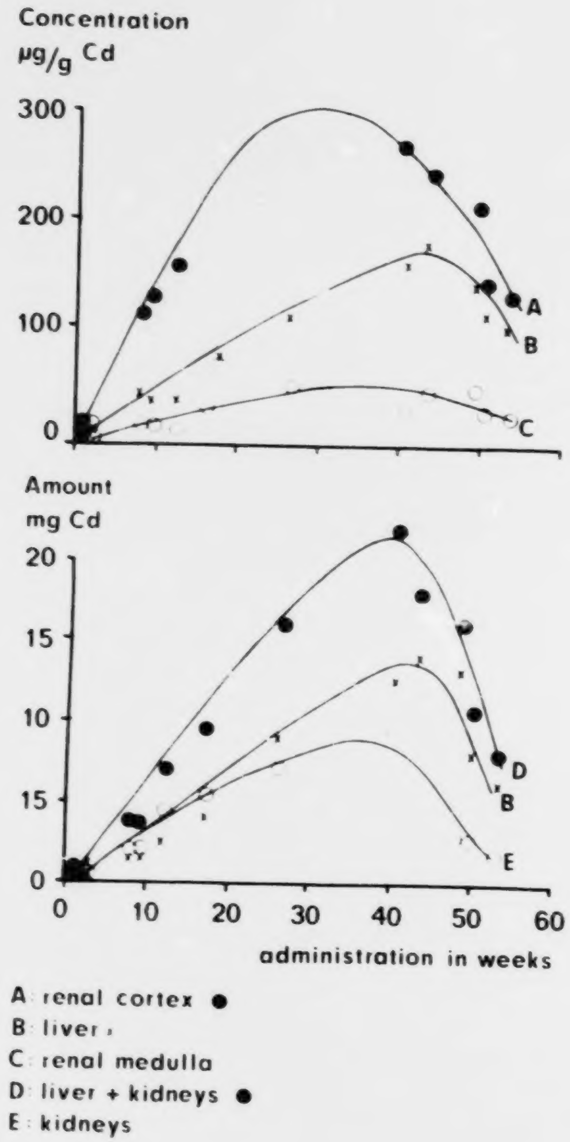

FIGURE 4:8 Cadmium in organs of rabbits orally administered 300 µg/g of cadmium (From Nomiyama, 1974).

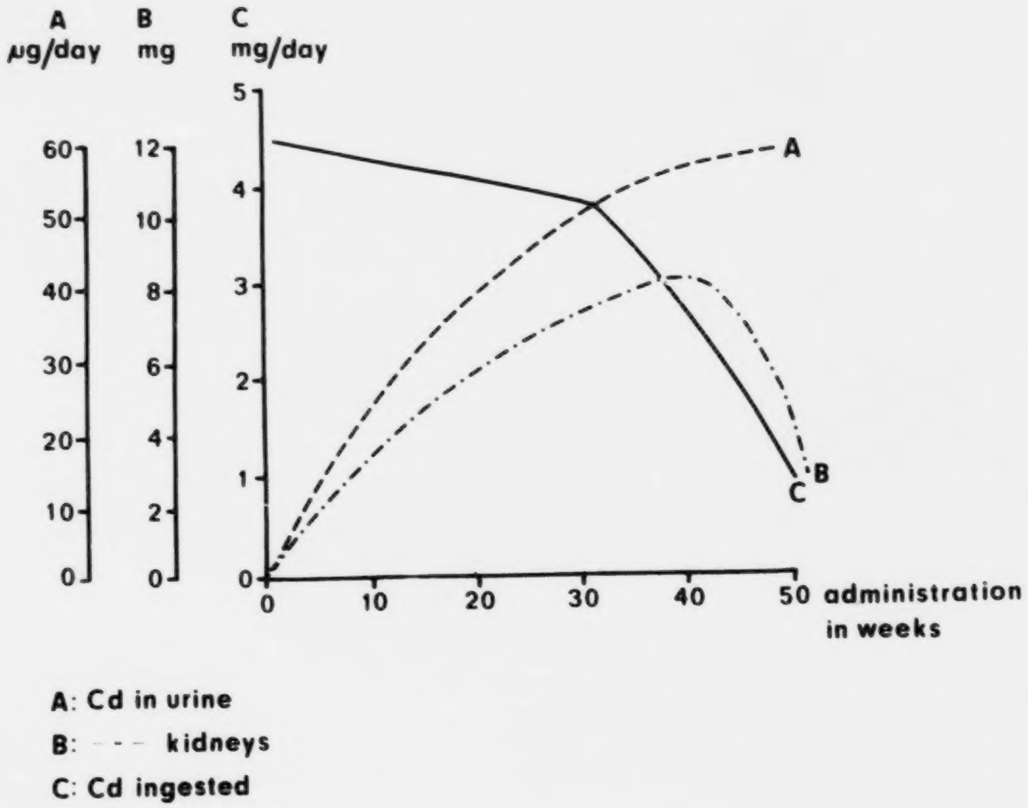

FIGURE 4:9 Relation between cadmium exposure and cadmium content in urine and kidneys of rabbits exposed to 300 µg Cd/g in food (From Nomiyama, 1974). Note: In the paper by Nomiyama, 1974, the intake at the beginning of the exposure was stated as 36 mg/day which is not consistent with his own data in this figure.

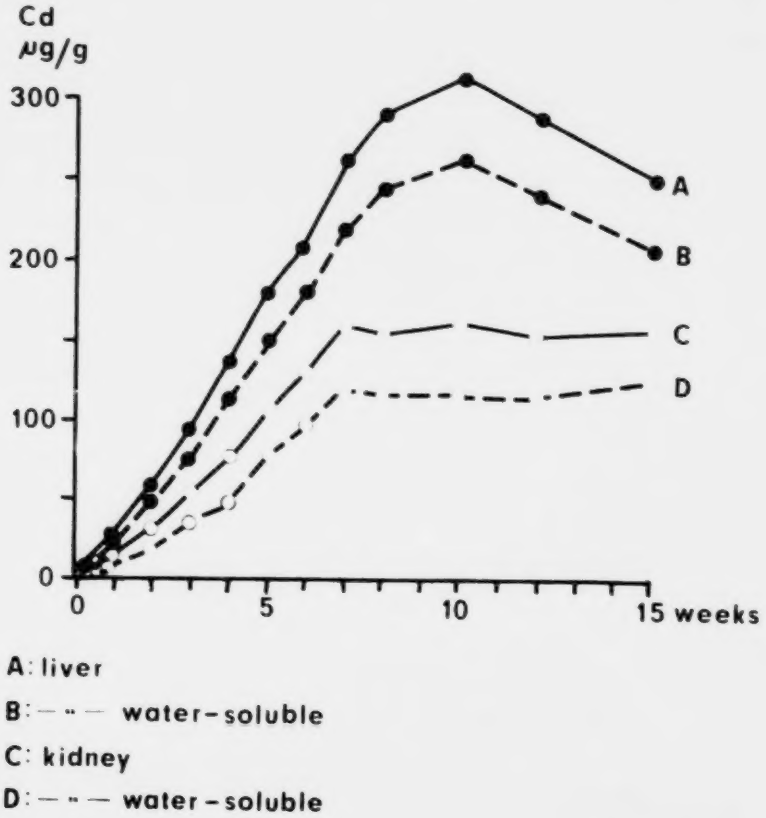

FIGURE 4:10 Total cadmium concentration in liver and kidney as well as in water-soluble fraction (protein-bound + ionized) of these two organs in relation to period of exposure (From Suzuki, 1974).

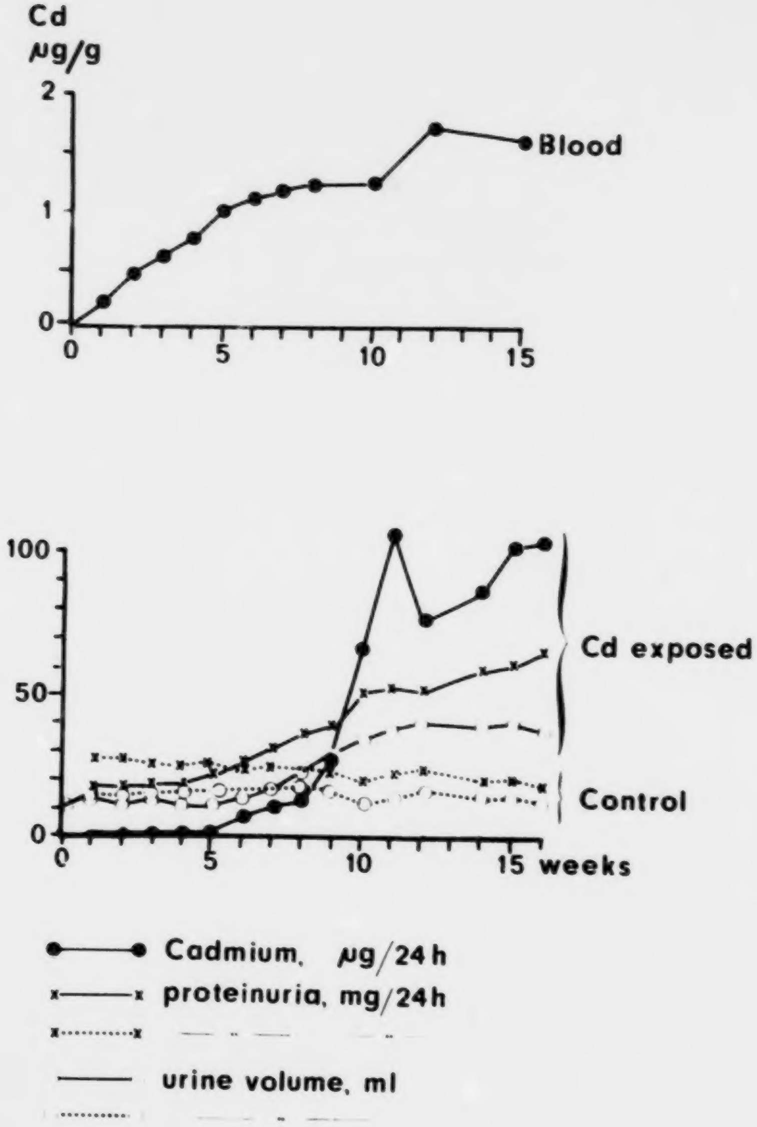

FIGURE 4:11 Cadmium in blood and urine as well as urine volume and proteinuria in relation to period of exposure. Average of 3 (blood) and 5 (urine) rats (From Suzuki 1974).

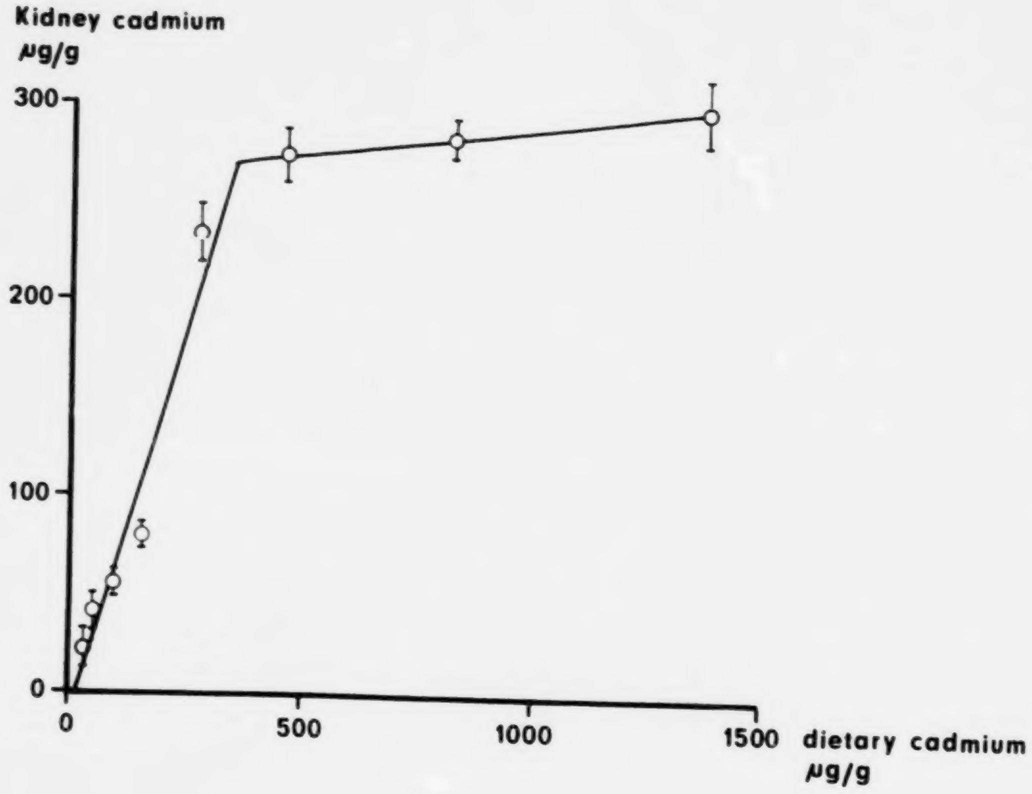

FIGURE 4:12 Regression of kidney cadmium concentration on dietary cadmium level. Means ± 95% confidence limits. Data from two experiments are included. The number of animals represented by each point are 4,4,4,4,4,3,4,4, and 3 for the 0,30,50,90, 150,270, 450, 810, and 1,350 µg/g Cd groups, respectively (From Cousins, Barber and Trout, 1973). Note: The cadmium concentration in kidney at 0-exposure was not given by these authors in their figure.

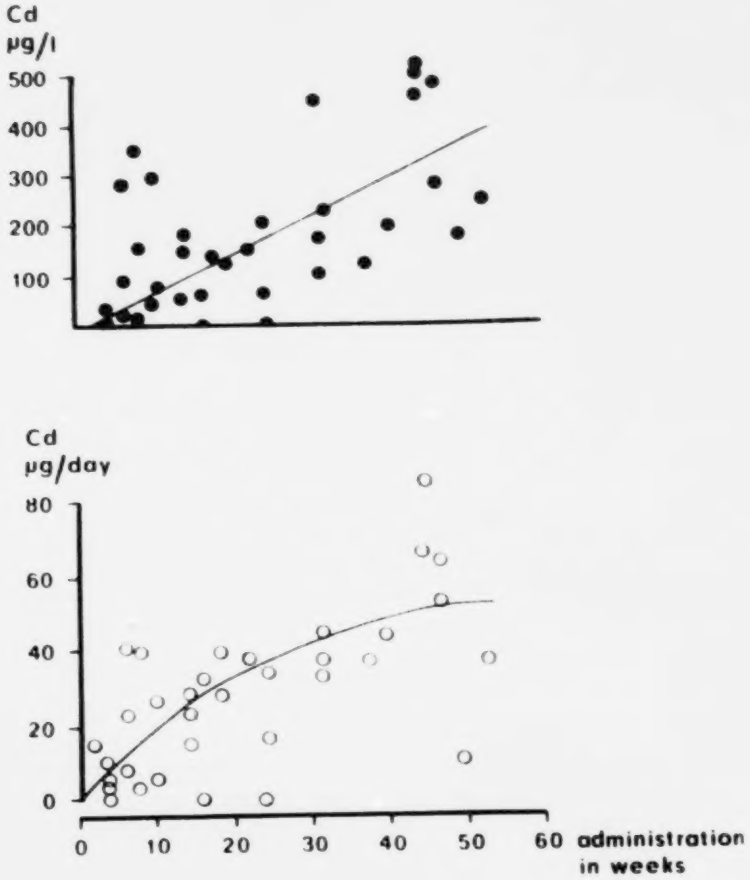

FIGURE 4:13 Excretion of cadmium in the urine of rabbits, orally administered 36 mq Cd/day (From Nomiyama, 1974).

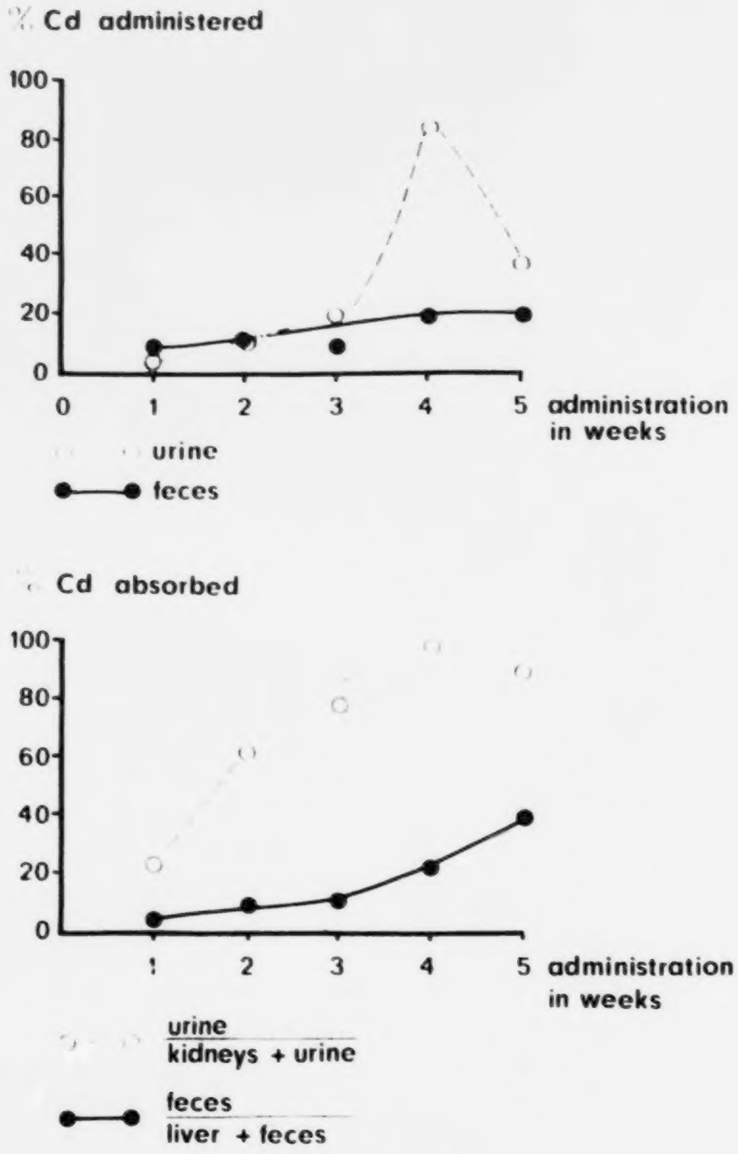

FIGURE 4:14 Percentage cadmium amount in urine and feces in relation to cadmium amount administered (above) absorbed into organs (below) (From Nomiyama, 1974).

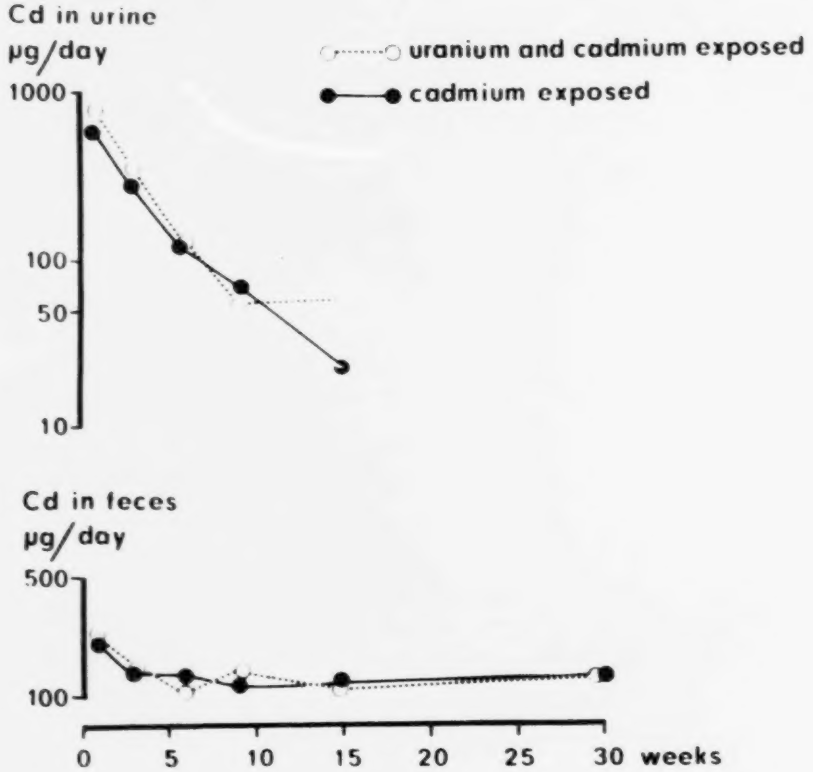

FIGURE 4:15 Excretion of cadmium in urine and feces after discontinuation of cadmium administration (From Nomiyama, 1974).

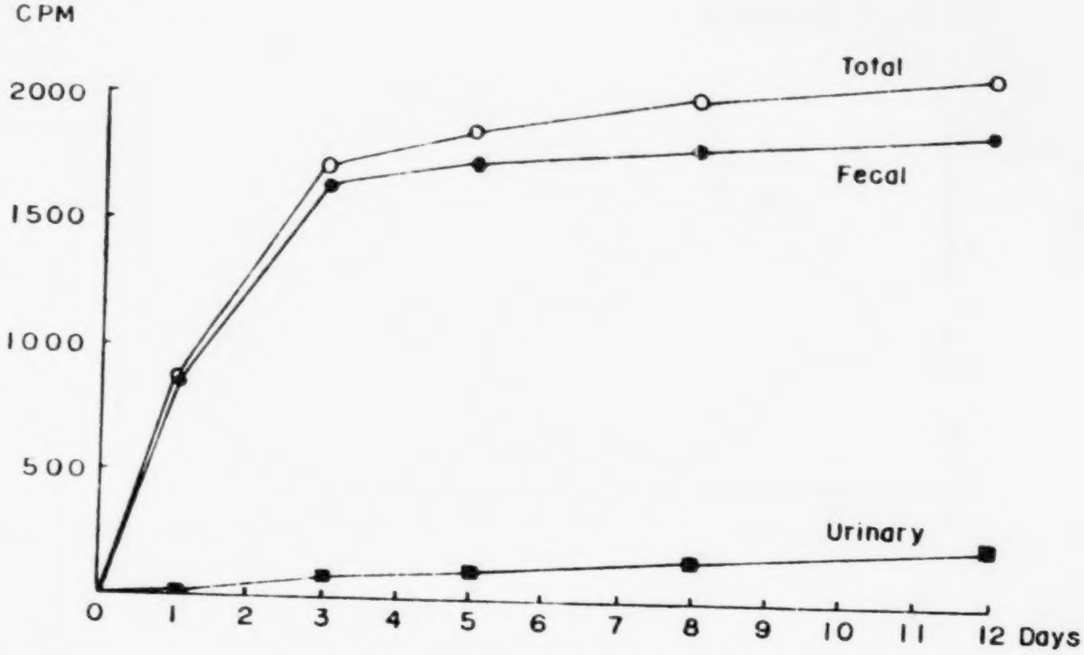

FIGURE 4:16 Excretion of $^{115m}CdCl_2$ from mice (From Ogawa, Suzuki, and Tsuzuki, 1972). Note: In their text, these authors stated that the daily excretion was shown in this figure. However, it is obvious that what is in fact depicted is the accumulated excretion.

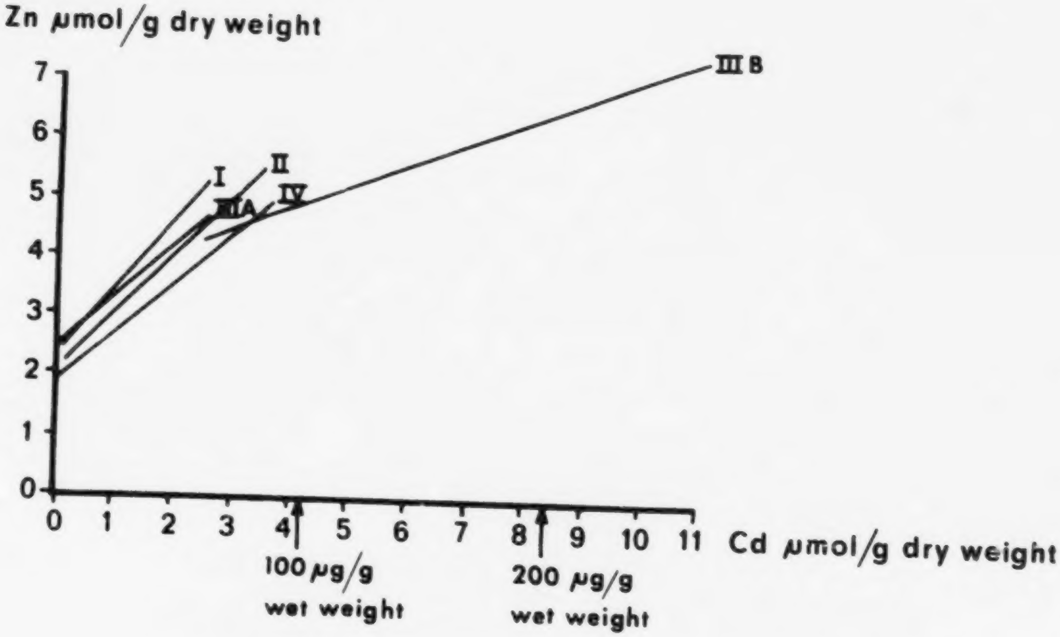

FIGURE 4:17 Regression lines for cadmium and zinc in renal cortex (From Piscator, 1974).

I Normal human beings, age 6-50 years, n = 36 (From Piscator and Lind, 1972).

II Normal human beings, age 10-90 years, n = 87 (From Hammer et al., 1973).

III Normal horses with A: cadmium concentration below 2.5 µmol/g, n = 20 and B: above 2.5 µmol/g, n = 17 (From Piscator, 1972).

IV Experimentally exposed swine, n = 12, whole kidney (From Cousins, Barber and Trout, 1973).

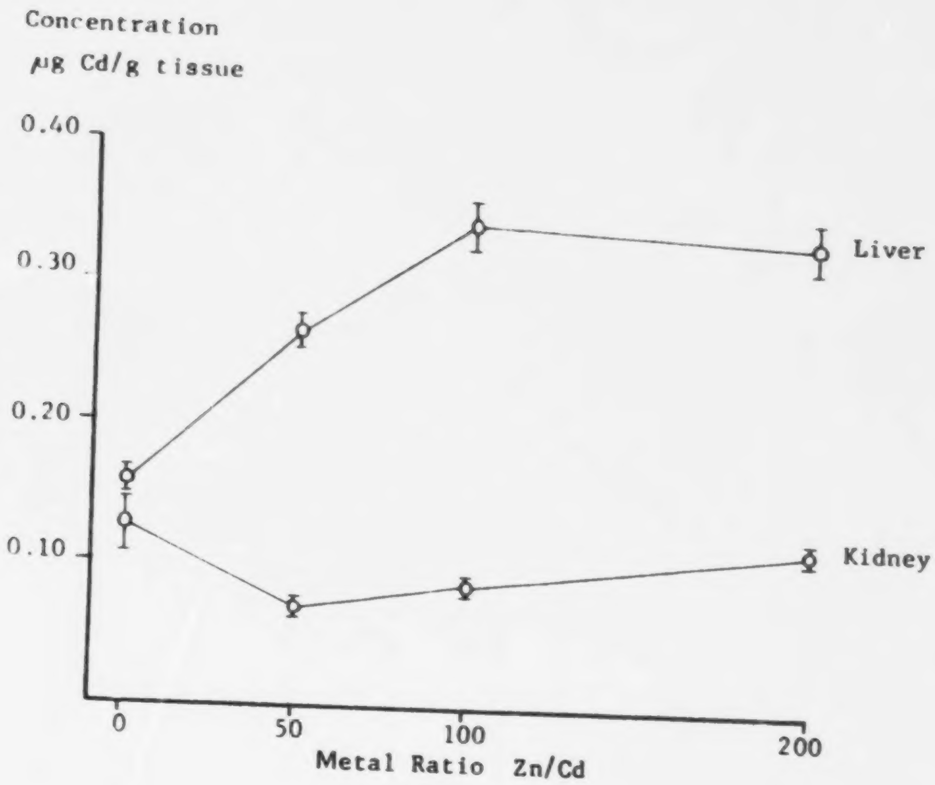

FIGURE 4:18 Influence of zinc on cadmium uptake (From Shank and Vetter, 1974).

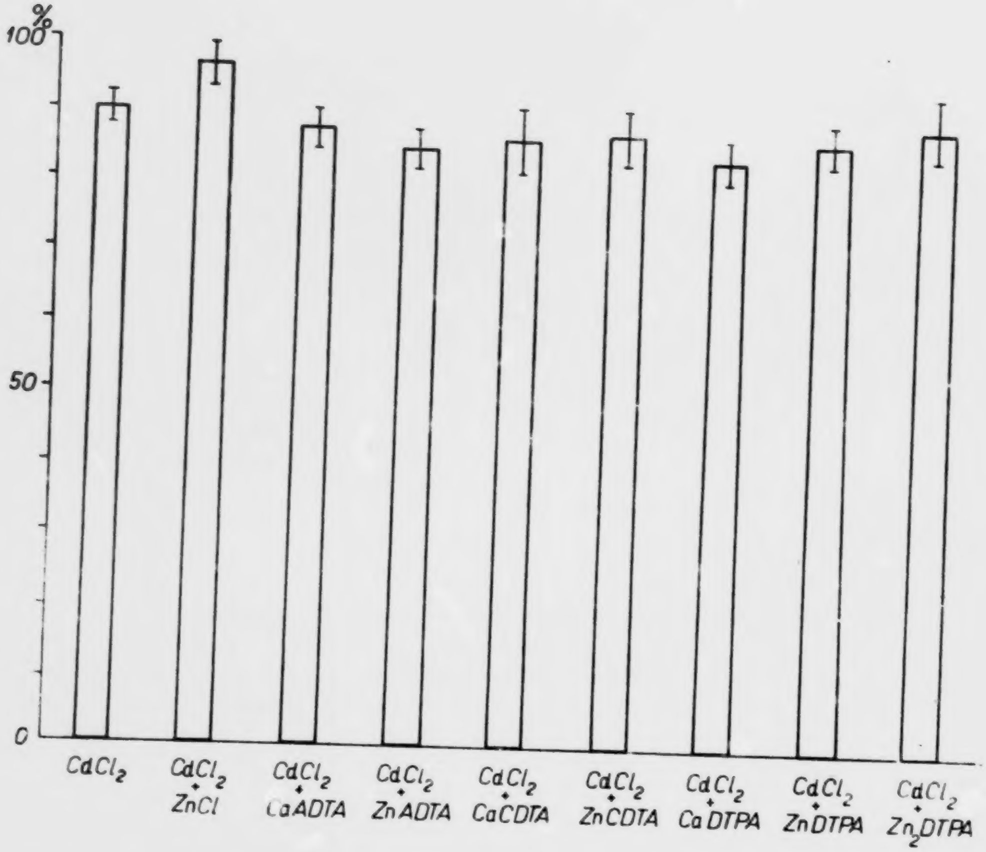

FIGURE 4:19 Influence of chelating agents on the whole body cadmium retention in mice in a subacute experiment. % = retention day 21 divided by day 7 (From Eybl, Sýkora and Mertl, 1974).

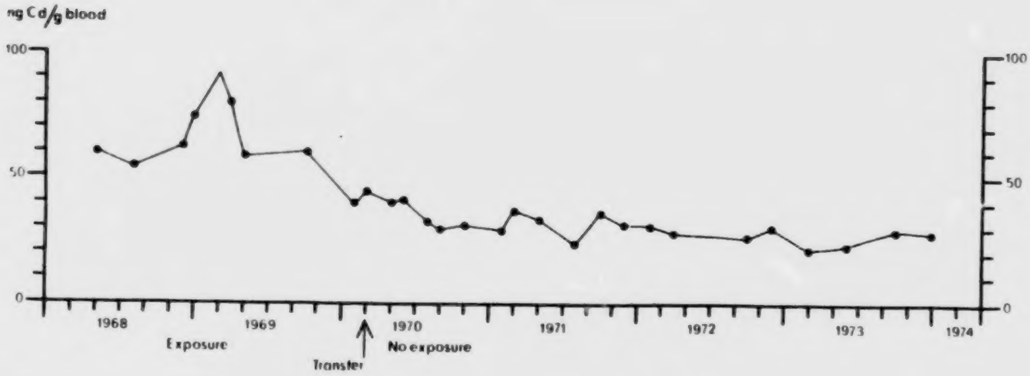

FIGURE 4:20 Cadmium concentrations in blood from a worker during and after exposure to cadmium oxide fumes (spectrographic method) (From Rogenfeldt, personal communication).

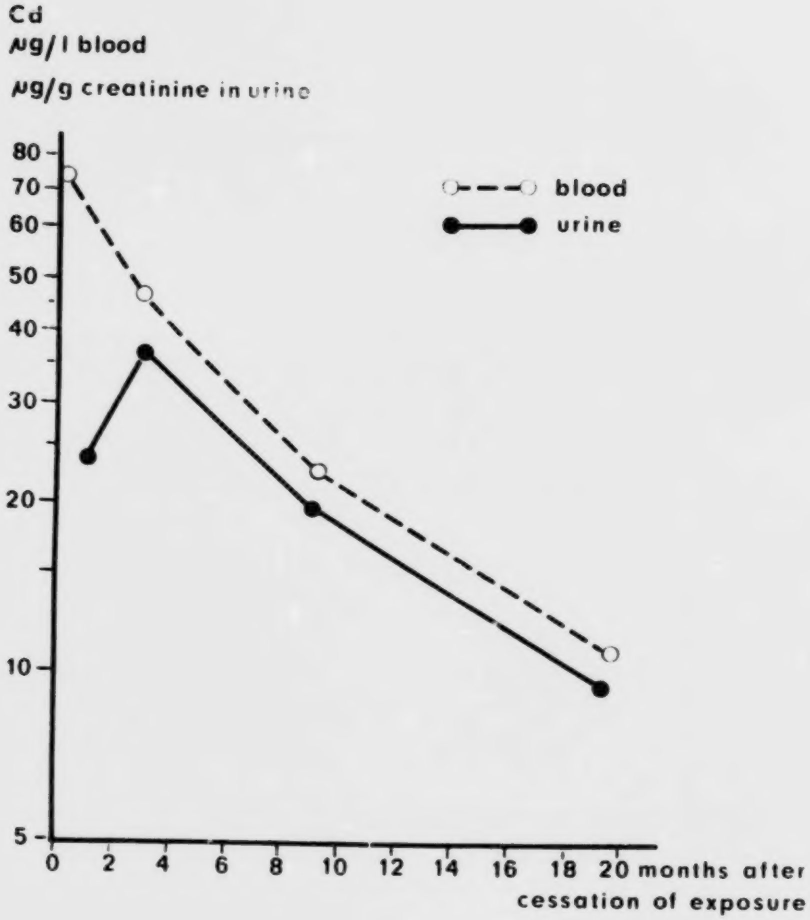

FIGURE 4:21 Cadmium concentrations in blood and urine after cessation of exposure in five workers with previous high exposure to cadmium (From Piscator, unpublished data).

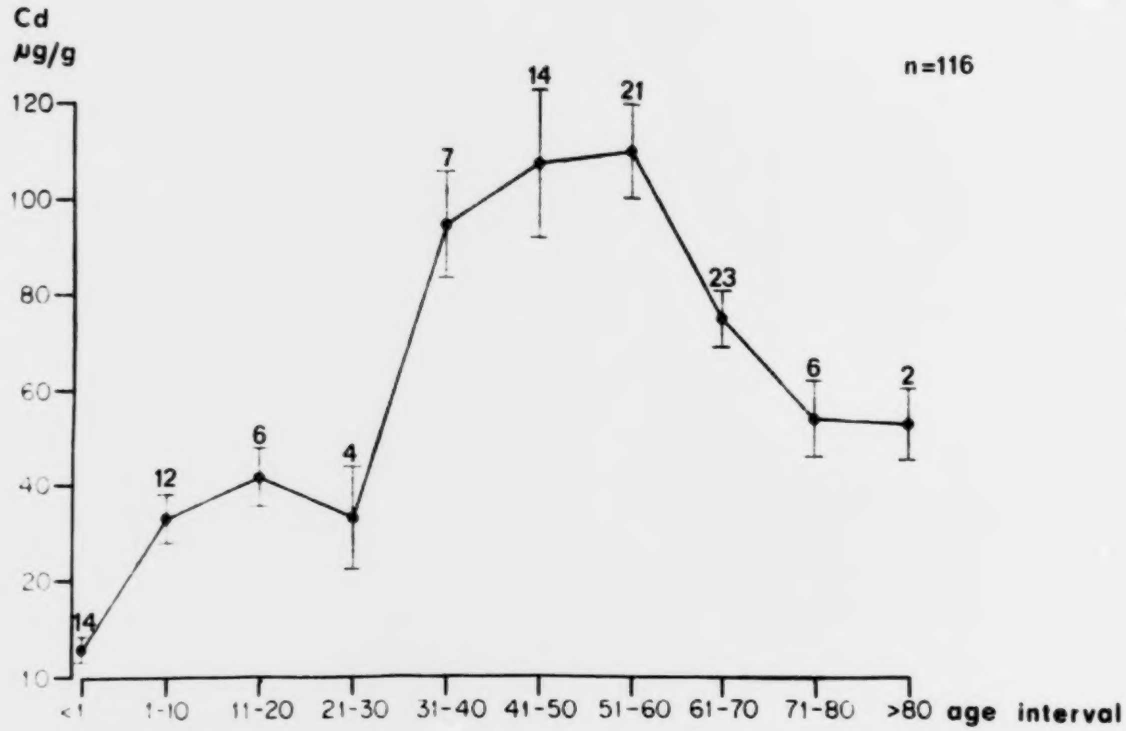

FIGURE 4:22 Relationship of kidney cortex content of cadmium to age.
Plotted are means : S.E.
Similar age relationships were noted for kidney cortex content of zinc, copper, lead and aluminum (From Indraprasit, Alexander and Gonick, 1974).

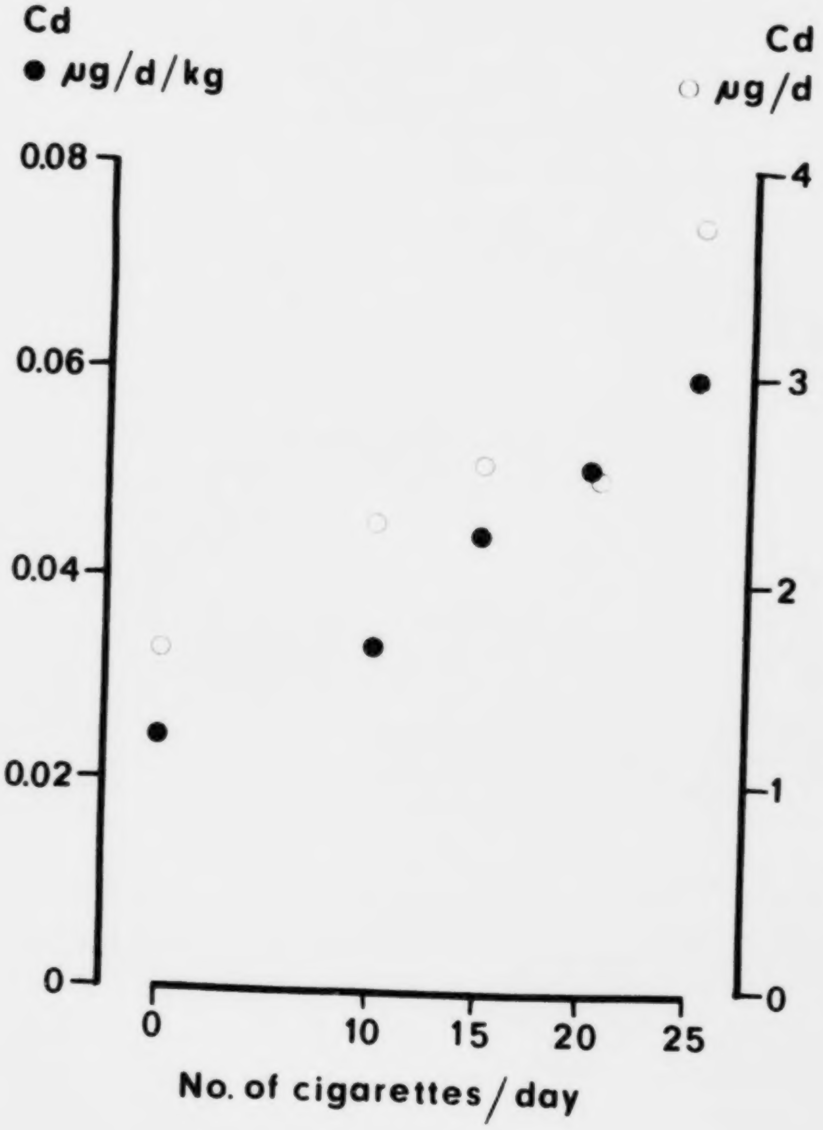

FIGURE 4:23 The average urinary excretion of cadmium for 5 days in 5 persons in relation to smoking habits (From Taguchi et al., 1972).

CHAPTER 5. EFFECTS

In CITE, 2nd ed., it was concluded that inhalation of high concentrations of cadmium compounds can give rise to severe, often fatal, pneumonitis, as shown both among human beings and animals. The estimated fatal dose for human beings was about 2,500 min. x mg/m^3 (corresponding to about 5 mg/m^3 for 8 hours). Acute effects among human beings after gastrointestinal exposure were also described. In animals the acute effects reported were studied mainly by injection of cadmium. Most organ systems could be involved, and the effects reviewed in CITE, 2nd ed., included hypertension and damage to the testicles, liver and kidney. The dose varied between 1-20 mg Cd/kg. Results as to acute effects show that cadmium is an extremely toxic metal, but they have only limited bearing with regard to the effects produced by long-term exposure.

Long-term inhalation of cadmium compounds can give rise to chronic pulmonary disorders, especially emphysema. It was concluded that cadmium oxide fumes may be more dangerous than cadmium oxide dust. A prolonged industrial exposure to well below 0.1 mg/m^3 as fumes may be considered hazardous with reference to emphysema.

Renal tubular damage has been shown as a chronic systemic effect of cadmium exposure, regardless of the route of exposure. The characteristic sign of the renal dysfunction is proteinuria of the so-called tubular type. This was considered to be due to decreased tubular absorption of filtered proteins. As cadmium seems to be transported to the kidney with a low molecular weight protein, metallothionein, the decreased reabsorption of this protein is thought to cause increased excretion of cadmium in connection with the renal damage. Other signs of renal tubular dysfunction in cadmium exposure are glucosuria, aminoaciduria and changes in the metabolism of calcium and phosphorus . It was concluded in

CITE, 2nd ed., that the critical concentration in the renal cortex for the development of tubular proteinuria was about 200 µg/g wet weight. In calcium deficient animals indications that the critical concentration might be even lower were pointed out.

Epidemiological studies in Japan have shown an increased prevalence of proteinuria in the areas most polluted with cadmium. It was concluded in CITE, 2nd ed., that cadmium played a causal role in renal tubular dysfunction in persons living in such areas.

Cadmium may cause a disturbance in calcium and phosphorus metabolism and osteomalacia has been observed in workers industrially exposed to cadmium. Animal experiments have shown demineralization of bone after cadmium exposure. In one highly cadmium-polluted area of Japan, renal tubular dysfunction combined with osteomalacia or severe osteoporosis had occurred endemically during the 1940's and the 1950's. The disease was called Itai-itai disease and in CITE, 2nd ed., it was concluded that there was no doubt that the disease was an expression of chronic cadmium poisoning. Deficient consumption of certain essential food elements and vitamins had been a contributing factor in the etiology, however.

Other chronic systemic effects found in animals and dealt with in the rundown of the cadmium literature given in CITE, 2nd ed., were hypertension, anemia, liver dysfunction, testicular changes and teratogenic effects. It was concluded that more data would be necessary to evaluate the yet unknown significance of these findings for human beings.

Studies have shown that cadmium and cadmium compounds could give rise to malignant tumors in rats at the site of injection. It was put forward in CITE, 2nd ed., that the data on carcinogenic and genetic effects were by no means conclusive and that further studies were necessary.

In the following treatise a strict division of the effects according to type has not been set up. Instead, a division has been made into studies on animals, highly exposed groups and general population. These divisions have been considered appropriate because many of the studies to be presented here confirm the earlier reported effects. In CITE, 2nd ed., studies in polluted areas in Japan were treated in detail in a separate chapter but here the new findings will be intermingled with other data on effects. Only new data of particular interest regarding these Japanese areas or the etiology of Itai-itai disease have been included.

We have emphasized effects on calcium- and vitamin-D metabolism, on the cardiovascular system and on testicles as well as teratogenic effects because these are matters on which original data are presently accumulating.

5.1 RESPIRATORY, RENAL, LIVER AND BONE EFFECTS

5.1.1 Studies on animals

5.1.1.1 Acute exposure

Snider et al., 1973, exposed rats one hour a day for 5-15 days to a cadmium chloride aerosol with a mass median diameter of 4.5 micron and a concentration of 10 mg/m^3. The animals developed acute pulmonary symptoms including alveolar hemorrhage. Ten days after exposure the changes had transformed into changes resembling human centrilobular emphysema. The results confirm earlier data on the high toxicity of inhaled cadmium aerosols as referred to in CITE, 2nd ed.

5.1.1.2 Prolonged exposure

Early signs of cadmium intoxication were studied by Nomiyama, Sato and Yamamoto, 1973, in rabbits given 1.5-15 mg Cd/kg body weight per day up to 45 days for the lowest dose. Several of the animals died during the experiments. The authors calculated the median lethal dose of cadmium to be 40-44 mg Cd/kg body weight. By means of stop-flow analyses in animals exposed to 6 mg/kg/day the authors found signs of renal tubular as well as glomerular damage. In the experiments with 1.5 mg Cd/kg/day the earliest sign of intoxication was an increased excretion of phosphatase in the urine, appearing earlier than the proteinuria.

Chronic cadmium poisoning in rats has been studied by Kawai and Fukuda, 1974, and Kawai, Fukuda and Kimura, 1974. Six groups of five male rats in each were given cadmium ad libitum in drinking water. One group (control group) received only tap water with no additional cadmium and four groups received 10, 50, 100 and 200 μg cadmium respectively in tap water and were studied during eight and one-half months. The sixth group received the dose of 200 μg/g cadmium in tap water but was studied longer, for 18 months. The results are summarized in Table 5:1 where it can be seen that the cadmium concentration in liver is increasing more or less linearly in relation to the total dose given. On the other hand cadmium concentration in kidney increases in five of the groups, including the one given 200 μg/g for eight and one-half months, but in the sixth group, followed for 18 months, a slight decrease is witnessed. This decrease is not statistically significant (Kawai, Fukuda and Kimura, 1974). According to the authors typical lesions of tubular atrophy with interstitial edema were found in the kidneys of the groups given 100 μg/g cadmium or more. Already at the dose level 50 μg/g slight lesions appeared in some of the animals. This low dose corresponded to an average kidney concentration of 38.4 μg/g which would correspond to a kidney cortex concentration of about 57 μg/g (see CITE, 2nd ed.). In the group in which typical tubular pathological findings occurred in all animals, the corresponding cadmium concentration in kidney cortex was about 150 µg/g. Decalcification and cortical atrophy of the femur occurred in the animals, these injuries being indicated in Table 5:1 by the decreasing cortical thickness in percent of the total femoral thickness. The calcium content also decreased at the dose level 200 µg/g in water. Histological findings in the bones were reported already in the group given 50 µg/g cadmium in drinking water.

Other types of effects were a widespread atrophy of exocrine parenchymal cells in pancreas in the rats given 200 µg/g in water. There was also some increase of the Kupffer cells in the liver at the dose levels 100 µg/g and above in water. The authors concluded that bone might be the most sensitive organ at this early stage of cadmium intoxication. A comparison of the histological findings in the kidney and the cadmium concentration supports the theory that cadmium excretion from the kidney will increase when the kidney damage is sufficiently severe.

Bone changes were found by Itokawa et al., 1974, who claimed that the changes were similar to osteomalacia in humans. Four groups of five rats in each were given the following diets and water:
Group 1: calcium adequate diet and distilled water (control group),
Group 2: calcium deficient diet and distilled water,
Group 3: calcium adequate diet and water containing 50 µg Cd/g, and
Group 4: calcium deficient diet and water containing 50 µg Cd/g.

Diet and water were provided ad libitum. The animals were studied for 120 days after which they were killed and blood and organ specimens were taken. Urine was sampled after 112 days.

The growth curves for groups 1 and 2 are very similar whereas those for group 3, and still more group 4, were significantly lower. Urinary calcium and phosphorus levels as well as red blood cell counts, hematocrit and hemoglobin values were significantly reduced in the cadmium treated groups.

The tissues of groups 3 and 4 showed hypertrophy and whitish--yellow color changes. A considerable degeneration of the kidneys occurred in these two groups. The greater part of the tubular epithelia was desquamated and vacuolized. However, necrosis and partial hyalinization in the glomerular capillaries and adhesions between the Bowman capsule and glomerular capillaries were also observed. Thinning of the cortical osseous tissue was seen in the bones of the calcium deficient rats. In the cadmium treated rats, fat deposition took place in the femoral spongiosa. In group 4, which underwent both calcium deficiency and cadmium exposure, there were also some osteoid borders on the bone trabeculae and an increased number of osteocytes in the cortical tissue. Itokawa et al., 1974, concluded that the bone findings were similar to the osteomalacia characterized by poor calcification as seen in humans.

A more subacute experiment, on rats given different combinations of high or low protein diet, high or low calcium diet, as well as cadmium in the diet was performed by Abe, Tanaka and Itokawa, 1972. The rats were studied for only four weeks and the cadmium given was about 200 µg/g in the food corresponding to a daily intake of 1.5 mg. After four weeks the average cadmium level in the kidney was about 200 µg/g dry weight and in liver about 150 µg/g dry weight. The bones were examined roentgenologically and the group given low protein and low calcium together with cadmium exposure showed an abnormal curvature of the spinal column. It is difficult to evaluate the actual pathophysiological changes in the bones.

Nomiyama, 1974, gave rabbits 300 µg Cd/g in food corresponding to a daily dose of about 36 mg Cd (sections 4.2.2 and 4.2.3.1). The earliest effects were an intermittently increased excretion of amino acids in urine after 16 weeks of feeding. At around the same time an intermittently increased excretion of enzymes such as alkaline phosphatase, GOT and GPT was recorded. Alkaline phosphatase in urine was significantly increased 3 times out of 16 between 14 and 52 weeks of exposure as seen in Nomiyama et al., in press (see section 4.2.3.1). GOT and GPT were only significantly increased during weeks 14 to 28 of exposure and not thereafter. Nomiyama, 1974, stated that hemoglobin was reduced after 27 weeks and that total protein and sugar excreted in urine reached elevated levels after 38 and 42 weeks respectively (see section 4.2.3.1). The cadmium concentration in kidney cortex at the time at which intermittent aminoaciduria developed was 200 µg/g wet weight whereas the total proteinuria and glucosuria developed at the concentration of about 300 µg/g. Nomiyama, 1974, claimed that no increased excretion of cadmium in the urine as a symptom of the kidney damage could be ascertained. It is doubtful whether such a conclusion can be reached in view of the obvious risk of contamination since the daily oral dose was 36 mg and the daily excretion 30 µg in urine and slightly less than 36 mg in feces (section 4.2.3.1).

In rats exposed subcutaneously to 0.5 µg Cd/kg body weight six days per week for 16 weeks, urinary protein excretion increased and urinary cadmium excretion increased dramatically (see Figures 4:10 and 4:11, section 4.2.2) at a calculated kidney cortex level of about 225 µg Cd/g wet weight (1.5 x 150) (Suzuki, 1974). These data confirm earlier data discussed in CITE, 2nd ed., regarding the relation of urinary cadmium to kidney damage.

Murase et al., 1974, studied the microscopic pathology of liver, kidney and some other organs in dogs that had received 500 ml of drinking water containing 100 µg Cd/g daily for 13 months. Cadmium in the cells was specifically stained with the Okamoto method. Two exposed dogs were compared with one control. There were hemosiderin deposits in the Kupffer cells and the liver blood vessels. An increased number of lymphocytes were discovered in the vascular regions of the liver as well as in the kidney, but in the kidney no changes were seen in the vessels themselves. There were pathological changes in about 30% of the glomeruli and about 25% of the proximal tubular epithelium.

No cadmium staining could be seen in the liver cells, but in the mesangium cells of the renal glomeruli as well as in the proximal tubular epithelium cells, fine cadmium stained granules were seen. These were diffusely spread throughout the cortex and in the same places, pathological changes in the cells were evident.

In CITE, 2nd ed., aminoaciduria was reported to be a common finding among cadmium-exposed workers and Itai-itai disease patients. As presented above, Nomiyama, 1974, found intermittent aminoaciduria to be the earliest indication of renal tubular damage by cadmium. Foulkes and Gieske, 1973, studied the specificity of metal effects on renal amino acid transport in a peracute study (20-60 seconds). The venous blood flow from rabbit kidneys was diverted into a vessel where samples could be taken at short intervals (seconds). By infusing labelled amino acids together with metal compounds as well as inulin, the inhibition of renal reabsorption of filtered amino acids could be measured. The authors reported that glutamate and aspartate reabsorption in the rabbits was specifically inhibited (24-68% inhibition) by an intraarterial cadmium dose of 2.5 µmoles Cd/kg body weight (0.28 mg/kg) given together with mercaptoethanol. Arginine reabsorption was inhibited 13-37% while the inhibition of phenylalanine, alanine, serine and lysine was lower or zero.

In conclusion it can be said that the recent studies on animals support the findings reported in CITE, 2nd ed. There are indications that histological changes in bones may develop earlier than renal damage but the data are not yet conclusive. It can still be said that the proximal renal tubules are the critical organs in chronic cadmium poisoning. Experimentally induced osteomalacia has been reported in cadmium exposed rats.

5.1.2 Studies on highly exposed groups

Metabolic aspects of the bone changes in chronic cadmium poisoning have been studied by Sano and Iguchi, 1974. They discussed the possibility that a decline in the formation of protocollagen or a disturbance of the hydroxylation of protocollagen would cause on the one hand an increased urinary excretion of proline and on the other hand an impairment of polymerization of soluble collagen. This would cause an accelerated decomposition of this type of collagen and increased excretion of hydroxyproline in urine (see Figure 5:1). Sano and Iguchi analyzed the excretion of free and bound proline and hydroxyproline in urine from 7 Itai-itai patients in the Fuchu area of Japan, 7 cadmium exposed persons in the Ikuno area, 5 cadmium workers and 10 control persons. Neither the exposure time nor the age of the persons were stated by the authors. As is seen in Table 5:2, a consistent increase in the excretion of these imino acids occurred among the Itai-itai patients from the Fuchu area. Most of the cadmium exposed persons in the Ikuno area, as well as the cadmium exposed workers, displayed a higher total imino acid excretion than the controls. The largest increases are in the excretion of free imino acids which in the first place might be a symptom of the decreased tubular reabsorption of these compounds, but the excretion of bound imino acid is also significantly elevated, especially in the Itai-itai patients, indicating the possibility of some abnormality in collagen metabolism.

Horstowa, Sikorski and Tyborski, 1966, presented the results of examinations carried out among 26 workers in Poland exposed to cadmium in the manufacture of alkaline batteries. Exposure time varied between 1-12 years and the exposure to cadmium in air was calculated as 0.13-1.17 mg/m^3. It is not mentioned in detail how the 26 workers were selected, but all had some form of signs which, according to the authors, could indicate chronic cadmium poisoning.

Proteinuria was detected in 7 out of the 26 workers, in 6 of these cases remaining after cessation of exposure. According to the authors, this indicated that the changes in the kidneys were persistent. The erythrocyte sedimentation rate was on an average 14 mm per one hour. Reduced vital capacity was found in 12 of the workers and emphysema in 17 upon X-ray. X-ray of the skeleton showed signs of osteoporosis in 10 cases. The occurrence of several other unspecific symptoms and signs is discussed. Difficulties exist in interpreting the paper, among other things due to the absence of controls and the nature of the methodology, which cannot be well understood without a knowledge of the basic Russian reference literature.

Lauwerys et al., 1974, reported on epidemiological studies of workers in Belgium exposed to cadmium in three different factories: an electronic workshop, a nickel/cadmium storage battery factory, and a cadmium-producing plant. In each factory a control group was selected to match the exposed group according to sex, age, weight, height, smoking habits and socio-economic status. Total dust as well as respirable dust (aerodynamic diameter $<$ 5 µm) was measured in the breathing zones of the workers. Each measurement covered about 4 hours and was repeated 4-5 times. The authors measured the cadmium concentration in the air only in connection with their present study, but were of the opinion that the said levels in the three plants were quite representative of past exposure, no important modifications having occurred in the different industrial processes since their installation. As always when no actual data exist on past exposure such a statement is open to discussion.

One group of 31 women (mean age: 30.5 years) was exposed for a mean period of 4 years (range: 1-12 years) to an average total and respirable (aerodynamic diameter $< 5\mu$) airborne cadmium dust concentration of 31 $\mu g/m^3$ and 1.4 $\mu g/m^3$, respectively (this group is in the article and here referred to as group E 1). The only difference upon comparison with the controls was a slight increase in the urinary cadmium concentration (5.3 $\mu g/g$ creatinine for the workers and 2.0 $\mu g/g$ creatinine for the controls). Blood values were 2.0 $\mu g/100$ ml and 1.0 $\mu g/100$ ml for workers and controls respectively.

Group E 2 consisted of 27 men (mean age: 38.6 years) exposed to an average total cadmium dust concentration of 134 $\mu g/m^3$ for 0.6-19.6 years (mean: 8.6 years). The average respirable cadmium dust concentration at the most polluted worksite was 88 $\mu g/m^3$. All pulmonary indices were on the average lower for the exposed than for the controls. None of the differences was significant, however. Average blood concentration of cadmium (3.0 $\mu g/100$ ml) among exposed workers was significantly higher than that of the controls ($p < 0.05$). In 4 out of the 27 workers electrophoresis of urinary proteins showed a pattern of glomerular proteinuria. Only one of the controls, known to suffer from glomerulonephritis, had proteinuria.

The methods used for electrophoresis of urinary proteins differed from methods employed in other studies. The urine proteins were concentrated to a volume corresponding to a creatinine concentration of 100 mg/ml, i.e. if the original creatinine concentration in urine was 100 mg/100 ml, the urine was concentrated 100 times. This means that if the original protein concentration was 10 mg/100 ml, the protein concentration in the concentrate would be only 10 mg/ml, which gives a very weak pattern in the agarose electrophoresis used in this study. Thus early tubular patterns may not have been recognized.

It is obvious that a positive finding by these authors mainly reflects a high protein excretion since at higher protein excretions in the original urine the concentrate will have a higher concentration. This is also borne out by the fact that electrophoretic patterns of urine proteins were not visible in the urine from two persons with 134 and 337 mg/g creatinine respectively, whereas from two persons with 851 and 1,254 mg protein per gram creatinine, clearly visible patterns were obtained (Figure 5:2).

Group E 3 consisted of 22 men (mean age: 51.5 years) exposed for 21-40 years (mean age: 27.8 years) to an average total and respirable cadmium concentration of 66 µg/m^3 and 21 µg/m^3 respectively. There was on an average increased cadmium concentration in urine and in blood. There were also changes in certain enzyme activities in blood and practically all pulmonary function tests carried out showed on an average pathologic values. Abnormal urinary protein patterns were revealed upon electrophoresis in 15 of the 22 urine samples from workers compared with none in the urine of the controls. The data are given in detail in Tables 5:3, 5:4, and 5:5. The excretion of cadmium in urine was also clearly related to the occurrence of proteinuria, as seen in Table 5:6.

In their discussion the authors concluded that the present TLV value is too high and recommended reduction of the TLV for cadmium dust to 50 µg/m^3. The groups studied by Lauwerys et al., 1974, would be very interesting to restudy in the future due to the careful collection of background data both among exposed and controls.

Tsuji et al., 1972, reported a study of 1,420 workers in the zinc refining plant of Annaka in Japan. Examinations for proteinuria, glucosuria and subjective symptoms were made on 1,398 persons. The group consisted of 1,247 men and 148 women who were divided into a low cadmium exposure group (619 persons) and a high cadmium exposure group (681 persons). A special study was also performed on 92 workers who were directly engaged in the production of cadmium. No control group was studied. Total average prevalence of proteinuria was about 15% when using the sulfosalicylic acid method and 6 1/2% and 9 1/2% in men and women respectively when using the trichloracetic acid method. Glucosuria (test-tape) was reported in 7.8% of the men and 1.4% of the women. In the group of workers directly engaged in cadmium production, the average prevalence of proteinuria (sulfosalicylic acid method) was 27 1/2% and 25% for men and women respectively. Among men over 50 years of age the prevalence was 50%.

The workers were examined for the yellow dental cadmium ring, but none of them had it. The workers engaged in cadmium production had significantly more respiratory tract and nasal symptoms than the other workers.

Tsuji et al., 1972, did not report on the cadmium exposure in the factory nor did they divide the workers into exposure time groups.

Nogawa et al., 1972, studied 283 painters of ceramics who were exposed to lead and cadmium. No control groups were studied. The urinary excretion of lead averaged about 70 ug/l in men and 50 ug/l in women whereas the cadmium excretion in men and women averaged about 6 μg/l. Cadmium excretion in urine showed a tendency to increase with age, but lead excretion showed no such tendency.

Proteinuria, glucosuria, hematocrit and hemoglobin were measured as effect indicators but no statistically significant differences between groups with different urinary cadmium excretion could be seen. On the other hand, when persons without proteinuria or glucosuria were compared with those with proteinuria or glucosuria, the cadmium excretion (expressed as μg/l urine) was significantly higher in the latter group in all age categories (up to 29 years, 30-59 years and 60 years or more). However, when cadmium excretion was expressed as µg/g creatinine, no statistically significant differences were seen.

Harada, 1973, and Harada et al., 1974, reported on a study of 19 workers in a cadmium pigment plant. No control group was studied but as seen in Table 5:7 a form of dose-response relationship between exposure time and proteinuria came forth. Proteinuria as measured by the trichloracetic acid method as well as by pathological patterns on disc electrophoresis hardly occurs at less than 5 years of exposure whereupon the prevalence increases. The air concentrations of cadmium were measured once in each part of the factory, the averages ranging from 24 µg/m^3 to more than 7 mg/m^3 (Harada, 1973). The author calculated that the worker with the longest exposure time and the most severe symptoms had been exposed to an average concentration of cadmium in workroom air of about 114 µg/m^3. The average concentration where most of the workers were stationed was estimated at 50 µg/m^3 (Harada, personal communication). The average urine cadmium excretion (µg/g creatinine, measured twice) ranged between 3.8 and 34.8 in those workers who did not have an increased globulin fraction on disc electrophoresis and from 23.4 to 130 µg/g creatinine in those who had a clear pathological increase of the globulin fraction on disc electrophoresis.

5.1.3 Studies on populations in Japanese cadmium-exposed areas

In CITE, 2nd ed., it was concluded that, in Fuchu area of Toyama Prefecture, the widespread occurrence of tubular proteinuria and the endemic of osteomalacia or severe osteoporosis in connection with such proteinuria were correlated to the level of cadmium exposure. Cadmium was seen as one of the etiological agents for these symptoms. In some of the other cadmium-exposed areas of Japan, proteinuria had also been regarded as an expression of cadmium poisoning of the general population. Some additional data from the areas mentioned in CITE, 2nd ed., will be dealt with here.

Shiroishi et al., 1972, studied 242 persons in S-village in Fuchu area of Toyama Prefecture as well as 161 persons in O-village (control area). It was not stated exactly how the target population was selected, but it consisted of 123 men and 119 women over 40 years of age in the S-village and 75 men and 86 women in the other village. Urine was studied for proteinuria with the Kingsbury-Clark method as well as with disc electrophoresis and for glucosuria with test-tape and the OTB-method. The occurrence of different patterns on disc electrophoresis is shown in Figure 5:3. The prevalence of Itai-itai disease pattern increases from about 10% in the 40-50 year age group to about 50% in the 70-80 year age group in this study.

In another study of 110 persons from the Fuchu area (Fukuyama et al., 1972) 24-hour urine samples were collected. Cadmium concentration was analyzed with atomic absorption after dithizone/chloroform extraction. Disc electrophoresis was also performed.In Figure 5:4 comparison is made between living time in the area and excretion of cadmium in the urine in the polluted area versus a control area. At around 40 years' time of residence, the cadmium excretion reaches its maximum, the prevalence of Itai-itai patterns on disc electrophoresis increasing drastically. After 45 years of residence in the area almost all persons in this study showed Itai-itai disease patterns and the average urinary cadmium excretion exhibited an age-related decrease.

Fukushima et al., 1973, reported on the cadmium concentrations in glutinous and non-glutinous rice as well as prevalences of proteinuria and glucosuria from villages within the Fuchu area. Non-glutinous rice is the type consumed as a regular food. The data from 37 villages are summarized in Figure 5:5. Response was measured as concurrent proteinuria (sulfosalicylic method) and glucosuria (Test-tape). As seen in the figure, response rate increases with increasing cadmium concentration in rice. The correlation coefficient for all 37 villages between response rate and rice-cadmium (non-glutinous) is 0.62, which is statistically significant.

The type of proteinuria among the Itai-itai patients and other cadmium exposed persons in the Fuchu area has been given considerable attention as accounted for in CITE, 2nd ed. Low molecular weight proteins like β_2-microglobulin and retinol-binding protein (RBP) have earlier been shown to be elevated in the urine among Itai-itai patients. This finding has now been given further support by Ohsawa and Kimura, 1973, as well as by Kanai et al., 1971. Kanai et al. also demonstrated that the increased excretion of RBP occurred among the relatives of Itai-itai disease patients. There was a distinct difference in the ratio between albumin and RBP among persons with Itai-itai disease as compared to persons with other renal diseases (Figure 5:6). Kanai et al., 1971, measured RBP with the single immunodiffusion method (Mancini). In later work they employed radioimmunoassay (Kanai et al., 1972), showing that the RBP level in serum of Itai-itai disease patients was not different from that of control persons. The increased excretion of RBP was concluded to be a symptom of decreased tubular reabsorption.

Utilizing isoelectric focusing, Vesterberg and Nise, 1973, showed a 4-60 times higher excretion of β_2-microglobulin in urine of 43 persons from the Itai-itai disease area than in the urine of 100 normal Swedes. Shiroishi et al., to be published, compared 10 Itai-itai disease patients, 32 observation patients (kidney symptoms but no bone symptoms, living in the Fuchu area) and 17 Japanese control persons. The increase in urinary β_2-microglobulin (radioimmunoassay) was about 200-fold whereas the increase of total proteins was only about 15-fold when the observation patients and the control persons were compared. There was no significant difference between Itai-itai patients and observation patients (See Table 5:8).

Nomiyama et al., 1973, studied the nature of urinary proteins in 7 female patients with Itai-itai disease. They found the molecular weights to be from 12 to 45 x 10^3, agreeing well with previous reports dealing with renal tubular dysfunction. Some of the low molecular weight proteins were retinol binding proteins and β_2-microglobulin proteins. There was some indication that the retinol binding protein was made up of the holo type to a greater extent than the apo type (holo type = RBP bound to vitamin A and apo type = free RBP) in Itai-itai disease patients as compared to cadmium workers with proteinuria. The authors speculated that the reason might be that Itai-itai disease patients have been treated with very large doses of vitamin A. There are very little actual data supporting a systematic difference between Itai-itai patients and cadmium poisoned workers in the paper.

From Ikuno area of Hyogo Prefecture some additional data are now available. Watanabe and Murayama, 1974, found no difference in the study of April, 1971, as to the average prevalence of proteinuria (trichloracetic acid method) between groups of villages with an average cadmium concentration in unpolished rice ranging from 0.33 ug/g up to 1.10 ug/g. In this same report, another study on 39 persons from a highly polluted area and 56 persons from a non-polluted area, in both cases randomly selected persons over 70 years of age, is discussed. In this study the prevalence of protein concentration above 10 mg/dl (as measured with a Biuret method) was 53.9% in the cadmium exposed group and 41.1% in the control group. In the same groups the prevalence of β_2-microglobulin concentration in urine above 10 ug/ml (Mancini method) was 41% in the exposed group and 3.6% in the control group. Lysozyme concentration in urine above 1 ug/ml occurred in 20.5% of the exposed persons and 3.6% of the control persons.

In conclusion it can be said that the newly available data from Japan further strengthen the conclusions reached in CITE, 2nd ed. The obvious possibilities of making large scale dose-response studies on early effects of cadmium in the general population have still not been realized.

5.1.4 Etiology of Itai-itai disease

Whether or not cadmium was one of the etiological factors for Itai-itai disease is still under intense discussion in Japan. Kajikawa et al., 1974, reported clinical and pathological findings in 11 cases of Itai-itai disease. The patients were autopsied between 1955 and 1961 and the results are summarized in Table 5:9. One patient had severe osteoporosis, two patients had primary hyperparathyroidism, and the other eight patients had osteomalacia at various stages of recovery. Kajikawa et al. concluded that the bone injury in Itai-itai disease is synonymous with osteomalacia. There is a difference between the two patients autopsied in 1955 and the six autopsied after 1965. The bone disease in the earlier patients was much more severe while in the later patients it was at different stages of recovery. In the kidneys of the two earlier patients chronic pyelonephritis and arteriosclerosis were found whereas tubular atrophy accompanied by interstitial fibrosis was found in the later patients. Kajikawa et al. reported that according to clinical examinations the later patients had a more severe damage of the kidney function and four of the six later patients died of uremia. The methods for analyzing proteinuria were not stated and no further clinical data were reported regarding the two earlier patients.

The advanced pathological changes in the kidneys must be hard to differentiate from the kidney findings in the other patients, hindering such a conclusion that the kidney damage was more severe in the later patients. Kajikawa et al., 1974, also studied autopsy findings in 155 patients who died from diseases other than Itai-itai disease. None of them had any known cadmium exposure. Of these patients 10 in the age range 26-79 years had tubular changes similar to those in Itai-itai disease. According to Kajikawa et al., the qualitative rating of "tubulopathy" in the Itai-itai disease patients was + to +++ and among the 10 other patients five had ±, three had + and two had ++. Seven of the 10 patients had died from cancer. These findings indicate that tubulopathy as diagnosed by Kajikawa et al. was an uncommon finding among

Japanese people dying from various diseases as compared to those dying with Itai-itai disease. On the other hand, 10 tubulopathies among 155 persons is not a low prevalence in itself. Kajikawa et al. concluded that it cannot be denied that the kidney findings among Itai-itai disease patients could be caused by chronic cadmium poisoning. On the other hand, they say that this kind of tubular damage has also been found among other patients in whom no increased cadmium exposure could be traced.

Apart from one of the Itai-itai patients, Kajikawa et al., 1974, could not find pathological signs in the kidney typical for vitamin D poisoning. Thus Kajikawa et al. maintained that it is very hard to conclude that the kidney damage in Itai-itai disease could be caused by vitamin D poisoning as had been suggested by Takeuchi, 1973. According to Kajikawa et al.,the kidney changes in the patients deteriorated over time whereas the bone changes improved. Vitamin D was effective in the treatment of the bone disease. Based on the observations they suggested that the bone findings in Itai-itai disease can hardly be caused by a cadmium-induced secondary vitamin D deficiency with osteomalacia. Instead, these authors believe the bone findings to be caused by malnutrition and vitamin D deficiency.

Kajikawa et al., 1974, also reported the cadmium concentration in various organs from seven of the Itai-itai patients and from one person who had lived in the polluted area and from whom they took biopsies during an appendectomy. The data from five of the patients are the same as reported by Ishizaki, Fukushima and Sakamoto, 1970. The cadmium concentration in the liver of the other two patients was 56.5 and 65.8 µg/g, similar to the values for the five patients earlier published by Ishizaki and associates. The person with appendectomy had a liver concentration of 41.8 µg/g. The concentration of cadmium in the kidney cortex was 17.9 µg/g in patient number 5 who had highly severe morphological kidney damage (see Table 5:9). Patient 11 had a renal cortex cadmium concentration of 123 ug/g. As seen in Table 5:9, this patient did not have any tubular nephropathy but only arteriosclerosis of the kidney. Patients 6,7,9 and 10 had similarly low renal cortex cadmium concentrations as patient 5 and as seen in Table 5:9 morphological changes were also more severe in the tubules than those of patient 11. These data support the theory that low cadmium concentrations in kidney of Itai-itai disease patients and workers with chronic cadmium poisoning were due to severe kidney damage.

Hagino, 1974, also discussed the etiology of Itai-itai disease but no new actual data were reported.

Hagino et al., 1970, reported the average urinary cadmium concentration in 9 patients of which 3 died. Twenty-four-hour samples were taken for about twenty days during two months. The average cadmium concentrations for the patients that were still alive were 12.9, 6.8, 24.5, 32.9, 7.3 and 16.1 ug/l. Among the three patients who died, the concentrations were 5.8, 7.7, and 9.9. In 1974, four years after the study, a fourth patient had died, i.e. the one having 6.8 at the sampling reported upon in 1970 (Hagino, personal communication). Hagino, 1974, concluded that patients with a severe case of Itai-itai disease and near death usually have lower cadmium concentrations in urine than other patients. According to him, this shows that cadmium concentration in urine cannot serve as an indicator of the exposure.

Recently much discussion in Japan has centered around the possibility that Itai-itai disease has occurred in areas other than the Fuchu area. As was mentioned in CITE, 2nd ed., authors have claimed that they have found at least 5 patients in the Ikuno area. More clinical data are now available; Nogawa et al., 1973, showed that one of the said patients had a syndrome very similar to Itai-itai disease, including tubular kidney dysfunction, increased alkaline phosphatase in serum, decalcified bones and multiple fractures. Some of these fractures were similar to the pseudofractures of osteomalacia. According to Nogawa et al., the average cadmium content in the rice harvested on the patient's farm was 0.64 µg cadmium per gram dry weight. This may correspond to a concentration of about 0.55 µg Cd/g wet weight.

Additional data about this and two other patients are given by Ishizaki et al., 1974. One of these patients had died and the cadmium content was analyzed in samples of tissues received at autopsy. The cadmium concentration in liver was 75 ug/g wet weight and the zinc concentration 116 ug/g wet weight. In kidney cortex the cadmium concentration was 53 ug/g wet weight and the zinc concentration 48 ug/g wet weight. Another organ with very high cadmium concentrations was the pancreas where 52 µg/g wet weight of cadmium and 56 ug/g wet weight of zinc were found. The cadmium content in liver, much higher than what is commonly found in Japan, was in the same range as for the Itai-itai disease patients in the Fuchu area. The cadmium concentration in kidney cortex was low in comparison with the liver value, probably due to the increased excretion of cadmium after kidney damage developed. Similarly low concentrations were also found among the Itai-itai patients in Fuchu area.

The scarcity of the data on individual Itai-itai disease patients, the lack of systematic follow-up studies on the patients, as well as dearth of autopsy data render the picture incomplete. Nothing that has come forth in the new data would influence the conclusions arrived at in CITE 2nd ed., that the Itai-itai disease is one form of chronic cadmium poisoning. The new data accumulating from the Fuchu and Ikuno areas on the contrary support the earlier conclusion.

Because of the small number of suspected cases found in Ikuno area it is not possible to say whether the syndrome is actually etiologically limited to cadmium exposure. The recent autopsy data, however, show that inhabitants of the area may be equally as exposed as those in the Fuchu area. Further research is needed into the prevalence of osteomalacia in the cadmium-exposed and control areas.

5.2 EFFECTS ON CALCIUM AND VITAMIN D METABOLISM

In CITE, 2nd ed., studies were reported that indicated an interrelationship between cadmium- and calcium- metabolism. Observations taken up there were that calcium-deficient rats have a higher cadmium uptake; a high prevalence of renal stones among cadmium workers suggested a disturbed metabolism of calcium and phosphorus. In addition, the effects of cadmium on bone were also reported as being secondary to effects on calcium-phosphorus metabolism. An impairment of the renal tubular regulation of Ca/P balance was proposed in CITE, 2nd ed., as the most important factor for elicitation of bone changes in workers.

The mechanism behind the Itai-itai disease has been arduously debated during recent years. Just as was the case for cadmium workers, cadmium was mainly linked to the osteomalacia by the epidemiological findings.

Sugawara and Sugawara, 1974, studied the influence that cadmium would have on calcium uptake from the intestine of rats. Three groups of 5-13 rats were given a diet ad libitum containing 0.22% calcium. In one group the food was mixed with a solution of cadmium in drinking water at the cadmium concentration of 10 µg/g and in another group cadmium concentration in drinking water was 50 µg/g. The rats were studied for 287 days and during days 3-24, days 45-66, days 161-182, and days 259-280, the total amounts of feces and urine were collected. During this time, the total food and water consumption was also measured. Calcium and phosphorus were analyzed in feces and in urine with atomic absorption method and the Allen method, respectively. Calcium and phosphorus absorption rates were calculated from the balance.

No significant differences in calcium or phosphorus absorption rates were seen between the control group and the group given 10 µg Cd/g in drinking water . There was a significant decrease in the calcium absorption rate to about half for the group given 50 µg Cd/g in drinking water and a decrease of about 25% in phosphorus absorption in the same group.

Sugawara and Sugawara, 1974, reported that in hematoxylin-eosin stained sections of the duodenum, pathological findings such as eosinophilic cell infiltration and erosions could be spotted in some of the rats given 50 µg Cd/g in drinking water. These authors suggested that the intestinal damage caused by cadmium leads to disorders of calcium metabolism. However, the only disorder shown in their study is the decreased uptake.

Studies into the effects of cadmium on calcium metabolism have also been reported by Kobayashi, 1973. Cadmium was mixed into a calcium-deficient diet in the amount of 30-100 µg/g. Rats given this diet then showed a negative calcium balance compared with rats on a calcium deficient diet without added cadmium. Figure 5:7 illustrates the calcium balance for individual rats, the positive numbers meaning that more calcium is gained from the diet than is excreted, and negative ones meaning that more calcium is excreted than was present in the diet. Kobayashi also noted that practically all the calcium excretion was via the feces. He therefore stated that a hypothesis that bone changes are caused by renal dysfunction was not supported. Instead, the postulation is favored that the loss of calcium is due to lower absorption of calcium and to a high excretion via the intestines.

The studies described above involved an exposure to cadmium via drinking water. The mechanism behind the effects on calcium uptake is therefore hard to evaluate.

The role of vitamin D in calcium metabolism has recently been reviewed by Kodicek, 1972. The active metabolite of vitamin D, 1,25-dihydroxycholecaliferol (1,25-DHCC), is formed in the kidney and stimulates calcium absorption from the intestine. A feedback control system including the calcium concentration in serum and parathyroid hormone (PTH) is involved in vitamin D metabolism. A schematic drawing of these pathways is shown in Figure 5:8. New data regarding the metabolism of vitamin D indicate that cadmium may have an inhibitory action on the enzymes necessary for the activation of vitamin D into 25-HCC and 1,25-DHCC.

In an experiment by Unger and Clausen, 1973, cadmium was administered by interperitoneal injection to groups of 2-10 mice. The dose varied between 0.1 ng Cd (as cadmium nitrate)/kg body weight up to 50 mg/kg body weight. The specific P-450 0-demethylation activity in liver microsomes was measured four days after the injection of cadmium. Table 5:10 reveals a type of dose-response relationship between decrease in P-450 activity and cadmium dose. The authors concluded that cadmium in vivo can affect metabolic transformations where the enzyme P-450 is involved, one of which is the transformation of vitamin D to 25-HCC.

Feldman and Cousins, 1973, studied the influence of cadmium on the synthesis of 1,25-DHCC in vitro in kidney mitochondria from chicks. The isolated chick kidney mitochondria were incubated together with solutions containing varying concentrations of cadmium chloride. Tritium-labelled 25-HCC was added and the metabolites formed after further incubation were separated by chromatography on a Sephadex column. The tritium content of each chromatographic fraction was measured with a liquid scintillation spectrometer. In this way it could be shown that increasing concentrations of cadmium depressed the formation of 1,25-DHCC (See Table 5:11). A similar experiment with other bivalent cations (Pb,Hg, Zn, Cu and S) with the same molarity showed that cadmium and lead depressed the formation of 1,25-DHCC in the most effective way.

Feldman and Cousins, 1973, also showed that chick cadmium-binding protein eliminated a large part of the inhibitory influence of cadmium on the chick kidney mitochondria formation of 1,25-DHCC. These effects of cadmium in vitro on vitamin D activation in the kidney have been confirmed by Suda et al., 1974.

Feldman and Cousins, 1973, studied the effects of cadmium in vivo on the renal vitamin D metabolism. 50 µg Cd/g in drinking water had been administered for two weeks to chicks given a low calcium diet. During eleven days they received intraperitoneal injections of 1mg Cd/day as cadmium chloride. After this time the chicks were killed and the hydroxylation activity in the isolated chick kidney mitochondria was tested. In the control chicks 50% of the substrate had been converted into 1,25-DHCC and in the cadmium-treated chicks only 30%. The extreme exposure resulted in a very high concentration of cadmium in the mitochondria, 770 µg/g of mitochondrial protein.

A similar experiment on the hydroxylation of 1,25-DHCC in the kidney was related by Kimura et al., 1974. Cadmium was fed with the diet in different concentrations to mice and the feeding time was slightly longer than in the experiment by Feldman and Cousins, 1973 (see Table 5:12). The production of 1,25-DHCC was slightly depressed in the groups given the highest doses. The difference in relation to controls was not statistically significant. (The number of animals was 2-3 in each group). In the group given the highest cadmium dose the concentration in total kidney was 36.5 µg/g wet weight. It may be that the cadmium concentration in kidney cortex was not high enough to induce large changes in the enzyme activity.

Cadmium-induced osteomalacia among rats has been reported by Itokawa et al., 1974 (section 5.1.1.2).

In conclusion the data reported above indicate that cadmium both in vitro and in vivo may inhibit enzyme activities which are important for the formation of the active metabolite of vitamin D in the kidney tubular cells. The data are still insufficient to explain the role of cadmium in bone mineral metabolism and further studies are needed.

5.3 EFFECTS ON THE CARDIOVASCULAR SYSTEM

It has earlier been claimed that hypertensive patients excrete more cadmium than normotensives (CITE, 2nd ed.). Wester, 1973, studied urinary excretion of cadmium in 16 hypertensive patients (11 men and 5 women, age 28-60 years) before and during treatment with chlortalidone (an anti-hypertensive and diuretic agent). Cadmium was determined with neutron activation. The mean daily excretion during 5 days before treatment was 2.1 µg and during 5 days' treatment 2.9 µg. This increase was statistically significant. The daily excretion in the hypertensives did not differ from that in normotensives. Cadmium was also determined in plasma. In 8 normotensive subjects a mean concentration of 2.5 ng/ml was found compared to 2.0 and 2.6 ng/ml in the hypertensive subjects before and during treatment. These results indicate that hypertensives do not differ from normotensives with regard to urinary excretion of cadmium. That chlortalidone caused an increase is explained by its chelating action. It can be mentioned that in the same study the excretion of zinc increased considerably during treatment.

Voors, Shuman and Gallagher, 1972, analyzed cadmium and zinc in different organs from about 100 autopsies from two areas in North Carolina, a rural area with high death rates in cardiovascular diseases, and an industrialized area with only moderate cardiovascular mortality. Information on smoking habits was obtained for about 64 % of the deceased persons by means of questionnaires filled in by their relatives.

Cigarette smoking was positively associated with kidney cadmium levels. Cadmium concentrations in the organs analyzed were also slightly higher in atherosclerotic than in non-atherosclerotic patients, although the differences were not statistically significant. The cadmium to zinc ratio in the kidneys was slightly higher in the atherosclerotic than in the non-atherosclerotic subjects (ratio: 0.68 and 0.55 respectively). The difference was statistically significant. The authors were cautious in interpreting their findings; the results may be a manifestation of the hypertensive process but increased cadmium levels could merely indicate increased exposure to environmental agents such as cigarettes, which were not possible to control for completely in the analyses.

Thind, Biery and Bovee, 1973, reported on the induction of arterial hypertension in 7 dogs after intraperitoneal injections of cadmium acetate (2 mg/kg body weight, about 25 injections during 36 weeks). Systolic blood pressure showed an increase of between 10 to 20 % on an average. There was no tendency to further increase in pressure after the sixth week. The authors did not use a control group, nor did they report any individual data. These gaps, plus the rather slight increase without dose relationships, make it impossible to evaluate the study.

Apart from the long-term study by Thind, Biery and Bovee there are studies in which different parameters, which might have a bearing on vascular effects, have been examined after single injection of cadmium compounds. In the brown bat, Youkilis et al., 1971, disclosed that a single intraperitoneal injection of 3 mg Cd/kg gave rise to small arterial dilatation while injection of 1 mg/kg cadmium caused small arterial constriction. Perry and Erlanger, 1973, found increased renin activity after injection of cadmium and mercury respectively. Thind, 1973, stated that cadmium and isoproterenol produce vascular relaxation (as studied on rabbit aorta strips). Massive doses of cadmium sulfate (2 mg/kg body weight intraperitoneally daily until 15 to 22 doses had been administered) were given to chickens by Sturkie, 1973. Compared with controls there was no increase in blood pressure, but a pronounced anemia was said to have arisen in the exposed animals. Toda, 1973, investigated the acute effects of cadmium ions on isolated hearts from rabbits and found several indications of an inhibitory action of the electrical activity of sinoatrial node pacemaker fibers.

During recent years a number of reviews on the relation between minerals and cardiovascular disease, particularly hypertension, have been published (Masironi et al., 1972, Masironi, 1973, Perry, 1973). As regards cadmium, the focus has been on the hardness of drinking water. Though some of the investigations have found an association between the two factors, it has been consistently stated that no proof of causality in human beings has yet come forth.

Perry, 1973, named three possible mechanisms by which cadmium might raise blood pressure. In acute hypertension induced by parenteral cadmium, the immediate increase in pressure is directly related to an increase in cardiac out-put. When chronic hypertension has been experimentally induced by cadmium, a corresponding increase in the circulating renin has appeared. The third mechanism may be related to the finding that repeated cadmium exposure induces a long term antinatriuretic effect.

5.4 EFFECTS ON THE TESTICLES

A vast literature has come to the fore concerning the acute effects of cadmium injection on the testicles and non-ovulating ovaries of various mammalian species, as has been pointed out in CITE, 2nd ed. Variation in susceptibility has been shown among species and even among strains of the same species. The reason for such variation has not been elucidated, nor has the exact biochemical, pathogenetic mechanism for the acute effect of cadmium on testicles, though several theories have been propounded. These two matters have been dealt with in many reports published during the last two years.

Sangalang and O'Halloran, 1972, 1973, have shown that the brook trout is extremely sensitive to minute cadmium concentrations in water. They reported that 50 % of a group of this type of fish died in 3 days, when exposed to a level of 5 ng/g of cadmium in 10 % sea water at 20°C. Hemorrhagic necrosis and vascular abnormalities of the testicles were not observed. When this type of trout was exposed to 25 ng/g of cadmium for 24 hours in soft fresh water (8-12°C) the testicles showed marked discoloration. Histological examination revealed extensive hemorrhagic necrosis and disintegration of lobule boundary cells. When exposed to 10 ng/g of cadmium, 7 out of 8 brook trout examined immediately post mortem showed similar injury, though less extensive. In vitro experiments with tissue from fish exposed to cadmium showed changes in testosterone metabolism in relation to control animals. The authors also performed several experiments with testicular tissue incubated together with cadmium in vitro. The results gave conclusive evidence that cadmium directly affects testicular steroidogenesis in the brook trout in vitro. The results of Sangalang and O'Halloran are also of interest in relation to the theory earlier advanced, namely that cadmium would only have a destructive effect in species with scrotal testicles. They showed that cadmium can also exert an effect on testicles of the brook trout, which has abdominal testicles. It seems thus, that scrotal testicles with their complicated pampiniform plexus, are not the only type of testicles affected by cadmium.

Abe, Itokawa, and Inoue, 1972,gave groups of mice consisting of 5-11 animals single intraperitoneal injections of cadmium chloride corresponding to 3 mg Cd/kg body weight. Six of the groups of mice were pretreated 24 hours earlier with intraperitoneal injections of 0.6 mg Cd/kg body weight, 5 mg manganese/kg body weight, 2 mg zinc/kg body weight, 1 mg vitamin C/kg body weight, 0.5 mg vitamin B_1/kg body weight and in the last group thiamin propyldisulfide (TPD). Seven days after the cadmium injection the animals were killed and compared with a control group consisting of 11 mice. Cadmium alone resulted in testicular atrophy and congestion as well as splenic hypertrophy, kidney atrophy and a decrease in hematocrit. Zinc and magnesium concentration in testis decreased whereas the zinc concentration in liver and the cadmium concentration in various tissues increased as compared to the control group. Pre-administration with cadmium could prevent the anatomical changes in the organs and the decrease in hematocrit was limited. The other pre-treatment procedures could not prevent the anatomical changes but the ability to prevent decrease of hematocrit was similar to that afforded by cadmium pre-treatment. The variations in metal concentration in tissues are hard to evaluate as the analytical method consisted of dry ashing of the samples, dissolution of the metals in the ash with diluted nitric acid and direct analysis of this acid in atomic absorption spectrophotometry without the use of deuterium background correction.

In a report by Sarkar and Mondal, 1973, it was shown that a single subcutaneous injection of cadmium chloride (5 mg/kg body weight, corresponding to 3 mg Cd/kg body weight) caused acute damage to testicular tissue in pigeon, and that this effect could be prevented by administration of zinc salt (40 mg zinc acetat per kg). This report too favors the assumption that not only the mammalian testicle, but also testicles of other species, are sensitive to cadmium, and that scrotal testicles are not necessary in order for the effect to occur.

It was mentioned in CITE, 2nd ed., that susceptibility to cadmium induced testicular necrosis was variable among strains of mice. In a study by Taylor, Heiniger and Meier, 1973, a genetic analysis of this resistance to cadmium induced testicular damage was performed. The pattern of resistant versus susceptible strains of mice (earlier reported by Gunn, Gould and Anderson, 1965) was confirmed, and by mating different types of strains, a dominance or partial dominance of susceptibility over resistance was demonstrated. From the pattern of resistant versus non-resistant strains (where there was a clear distinction between resistant and susceptible strains), the authors postulated that a single major gene may account for the difference in susceptibility. From further mating studies it was concluded that resistance to cadmium-induced testicular damage is determined by a single, recessive, fully penetrant gene. The strain distribution pattern of this gene shows that it is not another manifestation of some other known genes.

Dishon et al., 1972, have shown that urine of rats with ischemic or toxic (cadmium chloride induced) testicular necrosis contains testis specific antigenes. Liver and kidney specific antigenes were also excreted in the urine of animals injected subcutaneously with cadmium chloride (18-25 mg $CdCl_2$/Kg = 11-16 mg Cd/kg).

In a paper by Koskimies, 1973, the effect of cadmium on the protein composition of fluids in rete testis and seminiferous tubules has been studied. The author showed that serum protein concentration, mainly albumin, increased in the rete testis fluid already 10 minutes after cadmium injection. In the semiferous tubules, such an increase was not found until 2 hours after cadmium administration. The rapid rise of protein content of the rete testis fluid was considered to reflect changes in the permeability of the capillaries and/or epithelium there. The time difference between the reaction of the rete testis and seminiferous tubules supports the view that rete testis is the most vulnerable part of the intratesticular duct system.

A study was performed by Ray and Chatterjee, 1973, on the effects of reserpine and adrenalectomy on the action of cadmium on scrotal and cryptordial testes in the rat. Cadmium (cadmium chloride 4.5 mg/kg body weight: 2.8 mg Cd/kg body weight) was shown to have a clear effect on the weight of the cryptordial testicle whereas this effect was blocked by administration of reserpine or adrenalectomy. The authors suggested that a prerequisite for the cadmium-induced injurious effect on the vascular epithelium would be that appropriate amounts of catecholamine are available.

In CITE, 2nd ed., the two prevailing theories to explain the cadmium-induced testicular necrosis were outlined. These two theories rested upon 1. circulatory failure due to vascular damage, and 2. decreased utilization of cadmium. It was further evident from the discussion given in CITE, 2nd ed., that the majority of the researchers who have studied cadmium-induced testicular damage now seem to agree that the primary mechanism of cadmium action centers upon the vascular system and the capillaries in the testicles.

Lee and Dixon, 1973, gave an account of experimental findings with regard to cadmium distribution in the testicles as well as the effects of cadmium on such parameters as thymidine uptake into spermatogonial cells, incorporation of uridine and L-leusine into spermatides etc in cadmium exposed mice. They also had groups of mice exposed to combinations of cadmium and zinc. They interpreted their data as favoring a primary action of cadmium on the utilization of zinc by the spermatogenic cells, as well as an inhibition of DNA synthesis by spermatogonial cells.

The following methodological consideration may give their data a different interpretation. The authors mentioned that the distribution of cadmium in the testicles was studied by velocity sedimentation cell separation technique, as well as by autoradiography. With regard to the first mentioned technique, it is not possible to evaluate to what extent elongated spermatides and remnants of interstitial tissue, which will also be present in the preparations, could be separated. Such a differentiation is of importance, but a detailed discussion of this problem has not been given in the report, meaning a possibility that the fraction designated as "late elongated spermatides" also could have contained some material from the interstitial part of the testicle. With regard to the autoradiographic technique used by the authors, the following can be said: The method included fixation in Zenker-formol solution, and paraffin embedding and sectioning at 5 microns. The technique evidently was a completely wet technique including an acid fixative, providing abundant opportunities for cadmium to be dissolved and displaced in the specimens before the actual autoradiography was performed. The data thus cannot be considered reliable. The difficulties involved in connection with autoradiographical studies with cadmium have been pointed out by Berlin, Hammarström and Maunsbach, 1964, as well as by Nordberg, 1972 . The latter author showed that regardless of type of administration of cadmium, the cadmium in the testicles will be localized predominantly in the interstitial tissue of the testicles. Nordberg instead chose dry techniques and took care not to dissolve cadmium from the specimens before autoradiography was performed.

Concerning data on effects of cadmium also reported by Lee and Dixon, they are not conclusive with regard to the primary site of action of cadmium, and in view of the equivocal findings concerning the distribution, the paper cannot be said to give good support for the theory of primary action of cadmium on the spermatogenic epithelium. It must therefore still be considered most likely that the primary action is upon the vascular part of the testicles.

5.5 TERATOGENIC EFFECTS

Transplacental transfer of cadmium and the effect of cadmium on the placenta have been given comment in Chapter 4 of this report. It was concluded that the transplacental transfer of cadmium will probably be dependent on factors such as time of administration in relation to gestational age, route of administration and cadmium dose. After small oral or subcutaneous doses, cadmium does not accumulate in the fetus and the uptake in the placenta is small. When large intravenous injections of cadmium are given in the earlier parts of pregnancy, substantial transplacental transfer occurs, whereas in the latter part of pregnancy just prior to delivery, transplacental transfer of cadmium is negligible. When cadmium is administered late in pregnancy, the placenta is destroyed. A syndrome similar to a toxemia of pregnancy can be produced by cadmium injected during this last part of pregnancy. The following section will deal with teratogenic effects of cadmium only.

The first studies of the effects of cadmium on embryogenesis were performed on the golden hamster. Intravenous injection of cadmium into pregnant animals of this species during the 8th day of gestation brought forth teratogenic effects, especially on the facial architecture (Ferm and Carpenter, 1968). Cleft lips and palate as well as other types of facial defects were induced. The teratogenic effects of cadmium and other metals on mammalian embryos have recently been reviewed by Ferm, 1972.

Since the effects of cadmium, especially on testicles, have been shown to be counteracted by zinc, Ferm and Carpenter, 1968 also tested whether simultaneous injection of zinc could protect against the teratogenic effects of intravenously injected cadmium. The results are shown in Figure 5:9. It is evident from the figure that the simultaneous injection of zinc will protect against the teratogenic effects of cadmium. It has also been shown that cobalt protects against the toxic effects of cadmium upon sensory ganglia and testes (Gabbiani, Gregory and Baic, 1967). A similar protective effect of cobalt against the teratogenic effect of cadmium was tested by Ferm and Carpenter, 1968, but it failed to occur. The protective effect of zinc is not due to influence of zinc on the transplacental transfer of cadmium, since this transfer is not influenced by cadmium (Ferm, Hanlon, and Urban, 1969). Ferm, 1972, interpreted the observations as suggesting that cadmium acts directly upon embryonic tissue by interfering with zinc-metalloenzymes such as carbonic anhydrase and alkaline phosphatase.

When selenium is administered at or near the same time as cadmium, by intravenous injection in the golden hamster, it protects against the teratogenic effect of cadmium (Holmberg and Ferm, 1969). This protection by selenium occurs only when the time interval between the injection of the two metals is half an hour or less.

The simultaneous injection intravenously of inorganic salts of lead and cadmium to the golden hamster protected the embryo from the teratogenic effect of cadmium, and no cleft lips or palates were found in such embryos. On the other hand, cadmium appeared to potentiate the teratogenic effect of lead, producing more severe malformation of the caudal part of the embryo, a malformation typically produced by lead. Lead only, for example, produced only tail defects, whereas the combination of lead and cadmium produced embryos with severe malformation of the lower extremities including sympodia, as well as umbilical hernias. Neither of these types of malformations was noted when either of these two teratogens was used alone (Ferm and Carpenter, 1967, Ferm, 1969).

In a later report by Ferm, 1971, other congenital malformations were noted in the cadmium-exposed golden hamster embryo, such as limb defects including amelia and phocomelia. Further studies on the skeletal malformations resulting from cadmium treatment in the hamster were reported by Gale and Ferm, 1973. The technique followed was the one described by Ferm, 1971. The dose of cadmium was 2 mg/kg of cadmiumsulfate (3 $CdSO_4$ 8 H_2O) injected intravenously on the 8th and 9th day of gestation, corresponding to the time of major organ differentiation in the golden hamsters. Skeletal malformations were examined after staining with alizarin. Skeletal abnormalities were reported in the form of changes in the ossification center of the skull, vertebral column, ribs, hyoid bone and sternum. Skeletal malformations of the fore limb and hind limbs were also observed at a higher frequency in the cadmium injected groups compared to controls.

Mulvihill, Gamm and Ferm, 1970, have shown that the palatal clefts induced by cadmium in the golden hamster can best be explained by mesodermal deficiencies in the palate region. These abnormalities are characterized by deficiencies in formation and delays in bone ossification.

All of the above mentioned teratogenic effects of cadmium have been studied in the golden hamster, and induced by intravenous injection of cadmium. Chernoff, 1973, studied the teratogenic effects of subcutaneous injections of cadmium in rats. He used daily injections of 4-12 mg of cadmium chloride per kilogram of body weight (2.5-7.5 Cd/kg). The injections were given on 4 consecutive days beginning on day 13 to 16 of gestation. This treatment resulted in a dose-related rise in the fetal death rate, decrease in fetal weight and increase in the rate of anomalies, which included micrognathia, cleft palate, club foot, and small lungs.

Ishizu et al., 1973, gave pregnant mice subcutaneous injections of cadmium as the chloride in doses of 0, 0.33, 0.63, 2.5 and 5 mg/kg on the 7th day of gestation. A dose dependent relationship was found from 0.63-5 mg/kg of Cd, as seen in Table 5:16.

The no-effect dose with regard to malformation in this study was found to be 0.33 mg/kg.

Barr, 1973, has shown teratogenicity in two stocks of Wistas rats from i.p. injection of $CdCl_2$ 16 µmol/kg body weight (1.8 mg Cd/kg). There was a much more prominent effect on fetal mortality in the "S"-stock than in the "C"-stock of rats. The type of teratogenic effect observed also varied with stock of rats. For example, there was a 55 % rate of diaphragmatic hernia in the "S"-stock when injections were made on day 10 of gestation. The corresponding rate in "C"-stock was only 0.8 %. The types of teratogenic effects observed were completely different from those observed in the hamster.

Since all of the above mentioned investigations on rats and golden hamsters were performed by injection of relatively large doses of cadmium as a single injection or as a repeated administration for a few days duration, the effect may be classified as acute or subacute. It is known from experience with various other teratogenic agents that inter-species variation is large for teratogenic effects. The interpretation of the observed effects with regard to possible teratogenic effects in man is therefore difficult because of the limited number of species studied. An additional difficulty for the interpretation is the different mode and duration of exposure in relation to the human situation with regard to environmental exposure to cadmium, which includes mainly chronic or subchronic exposures. Acute oral and inhalation exposures to human beings also exist, but exposure by the injection route is not applicable to the human being.

Because of the different type of exposure, the experiments by Cvetkova, 1970, and Schroeder and Mitchener, 1971, are of particular interest. Cvetkova, 1970, exposed female rats via the respiratory route to cadmium sulfate (3 mg/m^3) during pregnancy. On day 22 of gestation examination of half of the material was performed. Fetal weight was lower than in controls, but no evidence of fetal mortality was found. The other half of the material was allowed to be born. The weight of the newborns was lower than that of controls. The offspring of the cadmium-exposed rats also displayed an increased perinatal mortality.

Schroeder and Mitchener, 1971, exposed mice (Charles, Rivers C.D. strain) to 10 µg/g cadmium in double deionized water. The controls were given deionized water without addition of cadmium. The diet contained the following elements (µg/g wet weight): zinc 23.2; copper 1.95; manganese 12.25; cobalt 0.18; molybdenum 0.45; selenium 0.056; and chromium 0.60. Five pairs of mice were taken at the time of weaning, placed in separate cages and given cadmium in drinking water continuously. The mice were allowed to breed as often as they normally would up to 6 months of age. At weaning time pairs were randomly selected from the first litter (F1A generation), from the second litter (F1B) and allowed to breed ad libitum to produce F2-generations. Pairs were selected at random from the first F2A litters and allowed to breed the F3-generation. Experiments were terminated when the strain was obviously dying out or when 3 generations had been weaned. Control animals were bred similarly. The results with regard to cadmium are seen from tables 5:13 and 5:14. It is seen that cadmium was toxic to breeding mice. Congenital abnormalities included sharp angulation of the distal third of the tail, this abnormality appearing in 5 litters, comprising 41 of 255 offspring (16.1 %) of the F1 and F2A generation. There were also 34 runts (13.3 %) in the offspring living beyond weaning, 87 deaths before weaning (30.5 %), 2 maternal deaths and 2 dead litters. Three of 5 pairs failed to breed in the F2B generation and the experiment was discontinued.

These data from Schroeder and Mitchener show that the type of abnormality induced by long-term oral exposure was different from those induced in earlier studies on hamsters and rats. They also showed a significant difference in comparison with their own controls for several parameters. These experiments as well as those of Cvetkova, 1970, are of particular importance, as stated above, because the type of exposure employed is similar to what could be met in the human situation. Consumption of drinking water or drinks prepared from water containing as much as 16 µg/g of cadmium for prolonged periods of time has been reported for school children in Sweden (Nordberg, Slorach and Stenström, 1973).

In short, teratogenic effects of cadmium have indeed been demonstrated. However, the majority of the experiments have been performed with injection of relatively large doses of cadmium over a few days, only a few experiments having involved long-term oral administration. The experiment by Schroeder and Mitchener, 1971, in mice, did indicate that relatively small concentrations of cadmium in drinking water could exert an effect. It is therefore important to continue to look for teratogenic and embryotoxic effects in inhalation and oral exposures in various experimental animals. In a study of women occupationally exposed to cadmium in the U.S.S.R. (Cvetkova, 1970) a decreased weight of babies was found. More such studies would be of great interest. Neither this study, nor the animal studies at hand, yet provide sufficient data for a detailed evaluation of the risks for embryotoxic and teratogenic effects of cadmium in man.

5.6 ACUTE SYSTEMIC EFFECTS OF CADMIUM INJECTION - STUDIES IN RELATION TO CHRONIC EFFECTS AND THE INFLUENCE OF METALLOTHIONEIN

In earlier parts of this report as well as in CITE, 2nd ed., various effects of acute and chronic cadmium exposure have been reviewed. By far greater emphasis has been placed on chronic effects because these effects may arise at relatively low daily dose levels, which may be comparable to actual environmental exposures. Acute effects in experimental animals after injection of cadmium salts have been given only brief mention with the exception of the somewhat detailed treatment on testicles. Although effects demonstrated by injection of relatively large doses of cadmium compounds may be of limited practical value, mechanisms of a fundamental interest to metal toxicology have been revealed while investigating them. These mechanisms with all probability will be of importance for the effects elicited by the cadmium levels met with in the environment. A brief discussion of these mechanisms therefore will be given in the following.

Acute systemic effects of cadmium (reviewed in the foregoing section of this report and in CITE, 2nd ed.) include transient effects on the cardiovascular system with changes of blood pressure, which have been reported after intravenous injection of cadmium in doses 0.02 to 2 mg/kg, testicular effects at dose levels of 0.1 to 2 mg/kg, effects on non-ovulating ovaries in young female rats, placental necrosis in pregnant mice and rats at dose levels 2 to 4 mg/kg and lesions in sensory ganglia at the same (2 to 4 mg/kg) dose levels. At larger doses (2 to 6 mg/kg) liver damage can occur, the higher of these doses being, however, in the lethal range. In this lethal range renal damage can occur, but it is of a different nature from that produced by low level long-term exposure.

Lesions caused by chronic exposure to cadmium are quite different from those produced by acute injection experiments mentioned above. Renal tubular impairment with excretion of low molecular weight proteins is considered to be the critical effect in long-term low level exposure to cadmium (see earlier chapters of this report and CITE, 2nd ed.). Such proteinuria, first described in industrial workers (Friberg, 1950), could be reproduced in experimental animals (Friberg, 1950; Dalhamn and Friberg, 1957; Bonnell, Ross and King, 1960; Axelsson and Piscator, 1966; Axelsson, Dahlgren and Piscator, 1968; Nordberg and Piscator, 1972; Nishizumi, 1972). It was not certain as to whether the acute effects of cadmium injection mentioned above could also arise after chronic exposure. With regard to the testicular damage, Nordberg, 1972 , when studying this question in detail, found no testicular necrosis after long-term exposure to cadmium although higher concentrations accumulated in the testicles at the same site (interstitially) as in acute exposure (Nordberg, 1971, 1972). Testicular damage thus does not seem to be a part of the syndrome of chronic cadmium intoxication at the stage when tubular proteinuria first appears.

Testicular damage. Nordberg, 1971, 1972 , performed a series of experiments to test a theory implying a protective mechanism of metallothionein against acute manifestations of cadmium intoxication. Doses of 1 mg Cd/kg or higher by the subcutaneous route gave rise to testicular damage in CBA mice (Nordberg, 1971). The cadmium concentration in the testicles at such exposure was about 0.3 μg/g. It was also shown (Nordberg and Piscator, 1972; Nordberg, 1972) that CBA mice exposed to cadmium (0.25 mg Cd/kg or 0.5 mg Cd/kg 5 days per week for half a year by the subcutaneous route) had evidence of renal tubular impairment after about 5 months of exposure. These mice underwent no testicular necrosis similar to the one observed in the groups given only a single injection of cadmium (1 mg/kg), in spite of much higher concentrations of cadmium (6-7 μg/g) being present in the testicles. A possible explanation for the difference in tolerance of testicular tissue between acute and chronic exposure was the binding of cadmium to metallothionein. Nordberg, 1971, made the following experiments to elucidate this question:

1. In mice given pretreatment by 12 daily subcutaneous doses of cadmium chloride (0.25 mg Cd/kg) prior to a larger subcutaneous dose (1 mg Cd/kg), all animals had histologically normal testicles whereas mice given 1 mg Cd/kg, but no pretreatment, had testicles which were severely damaged. The protection offered by pretreatment is in accord with the theory implying metallothionein as a protective agent.

2. Two groups of animals, one group pretreated as stated above and one non-pretreated group, were both given a single subcutaneous dose (1 mg Cd/kg body weight) of cadmium chloride tagged with ^{109}Cd. By centrifugation and gel chromatography it was found that a larger part of the radioactivity from the 1 mg/kg dose was incorporated into a protein of the size of metallothionein in the pretreated animals than in the non-pretreated ones.

3. In another experiment the same total subcutaneous dose (1.1 mg/kg) of cadmium was given to different groups of animals. The dose was divided into different proportions between metallothionein-bound cadmium and cadmium chloride in the various groups of animals. In such groups given 0.4 or 0.6 mg cadmium per kg body weight as metallothionein-bound cadmium the testicles were usually normal. In groups of mice given only 0.2 mg Cd/kg as metallothionein-bound cadmium (and 0.9 mg Cd/kg as cadmium chloride) moderate to severe damage was seen. In mice given no metallothionein-bound cadmium (given 1.1 mg Cd/kg as cadmium chloride) the testicles were completely damaged.

In summary it may be said that Nordberg, 1971, 1972 has shown that metallothionein is involved in the protection of the testicles against necrosis in more prolonged exposures. It is likely that metallothionein acts similarly with regard to other acute effects of cadmium, examples of which have indeed been put forth by several authors. However, metallothionein may also be involved in elicitation of damage in long-term exposure to cadmium (see section 4.4).

Liver effects. It was shown already by Piscator, 1964, that cadmium in the liver of experimental animals exposed to this metal is to a large extent bound to metallothionein.

This has later been confirmed by a number of authors (Shaikh and Lucis, 1969, 1970; Wisniewska-Knypl and Jablonska, 1970; and Nordberg, Piscator and Lind, 1971). Nordberg, Piscator and Lind, 1971, reported markedly decreased liver weights and death in mice given 3 mg Cd/kg body weight as cadmium chloride. These effects were discussed in relation to the appearance of cadmium binding to metallothionein in the liver.

Further data relating effects of cadmium on the liver and binding of the metal to metallothionein have been supplied by Winge et al., 1973. These authors gave groups of animals 0.5, 1, 2, 2.5, 3, 4 or 6 mg Cd/kg body weight as cadmium chloride daily for 6 days. Animals receiving 0.5 and 1 mg Cd/kg exhibited no morbidity or mortality whereas animals in the 2 and 2.5 mg Cd/kg groups showed obvious morbidity. 2/3 of the animals receiving 3 mg/kg died by the 6th injection day while all of those given 4 mg Cd/kg or more died. Surviving animals were all killed on the 6th day whereupon the livers were examined by histological techniques and by gel chromatography. At injection levels of 0.5 to 1 mg Cd/kg cadmium was discovered to be bound almost exclusively to metallothionein. As the injection dose was increased, more cadmium accumulated in the liver, and protein fractions of high molecular weight acted to bind cadmium. The histological examination revealed no liver abnormalities in animals of the 0.5 and 1 mg Cd/kg groups. In the 2, 2.5 and 3 mg Cd/kg groups degenerating hepatocytes were seen. The authors concluded that non-metallothionein-bound cadmium was correlated to hepatic damage. As in the studies by Nordberg, Piscator and Lind, 1971, an evident correlation between clinical morbidity and absence of binding to metallothionein was witnessed.

Unger and Clausen, 1973, found a dose-related inhibition of cytochrome P-450 activity after a single i.p. injection. The authors noted that the effect diminished when the interval between injection and study of enzymatic activity was increased. This was ascribed to the protection offered by synthesized metallothionein.

Effects on glucose tolerance. As stated in earlier parts of this report, the pancreas accumulates considerable amounts of cadmium (Friberg, 1957; Smith, Smith and McCall, 1960; Ishizaki, Fukushima and Sakamoto, 1970; Nordberg and Nishiyama, 1972). Data on pancreatic functions in long-term exposure are sparce. Murata et al., 1970, have reported changes in the excretory function of the pancreas in Itai-itai patients. More recently, Ghafghazi and Mennear, 1973, reported on effects of acute and subacute cadmium administration on carbohydrate metabolism in mice. It is known from earlier studies (Friberg and Odeblad, 1957) that cadmium is equally concentrated in the exocrine and endocrine parts of the pancreas. Ghafghazi and Mennear, 1973, injected 2 to 6 mg/kg of cadmium acetate intraperitoneally to mice and observed a dose-related increase in blood glucose concentration. Administration of cadmium to adrenalectomized animals failed to evoke hyperglycemia, showing that this effect of cadmium is mediated through the adrenal glands. The same investigators also learned that the glucose tolerance was diminished after cadmium administration in the mice with adrenal glands as well as in adrenalectomized mice. Glucose intolerance was shown to be associated with a decreased pancreatic secretory activity of insulin measured as serum immunoresponsive insulin. The administration of 4 mg/kg of cadmium daily for 4-14 days produced resistance to cadmium-induced hyperglycemia. This subacute treatment did not produce changes in resting blood glucose levels nor did it produce a decrease in glucose tolerance. A significant reduction in circulating serum insulin was detected after subacute cadmium administration, which may be related to an increased renal excretion of glucose. The resistance against hyperglycemia built up by the repeated treatment was explained by the authors as stemming from the induction of metallothionein by the acute doses. They postulated that this protein prevented the effects on the pancreatic isles. This may be in accord with the observation that the main part of the pancreatic cadmium in mice during long-term exposure to cadmium was in the metallothionein fraction (Nordberg and Nordberg, 1973).

It has been mentioned previously that it is not possible to induce renal tubular effects by injecting a single large dose of cadmium. By injection of repeated large doses over a limited period renal effects can be induced, but these effects differ morphologically from those observed after more long term dosage of smaller daily doses of cadmium (Kawai, Fukuda and Kimura, 1975).

Renal effects. Studies of the influence of cadmium on the synthesis of 1,25-dehydroxycholecalciferol in vitro by kidney mitochondria have been described previously (Feldman and Cousins, 1973, section 5.2). These authors as well as Kimura et al., 1974, showed that metallothionein prevented the inhibitory effect of cadmium upon this process.

By combining cadmium injection with various chelating agents like 2,3-dimercaptopropanol (BAL) (Gilman et al., 1946, Tepperman, 1947; Niemeier, 1967) and ethylene diaminetetraacetic acid (EDTA) (Friberg, 1956; Eybl, Sykora and Mertl, 1966), an increased renal toxicity and an increased renal uptake of cadmium may be induced. Injection of a cadmium-cysteine complex (Gunn, Gould and Anderson, 1968) can induce a selective renal tubular injury and a prominent accumulation of cadmium in the kidney.

Recently Gieske and Foulkes, 1974, administered a mixture of cadmium chloride (10 μmol Cd/kg = 1.1 mg Cd/kg) and mercaptoethanol (350 μmol/kg) intravenously to rabbits and studied the resulting renal damage by various renal function tests. Glomerular filtration rate, p-aminohippurate clearance (C_{PAH}) and extraction of p-aminohippurate (E_{PAH}) were severely depressed in animals given Cd-mercaptoethanol. The filtration fraction (GFR/plasma flow) also decreased as well as reabsorption of amino acids. The site of inhibition of amino acid reabsorption was considered to be the luminal membrane of the tubular cells. On the other hand the tubular ability to reabsorb gluocse was unaltered when corrected for changes in GFR, thus indicating that the effect of Cd-mercaptoethanol on the kidney was relatively specific.

Since mercaptoethanol does not occur in appreciable amounts in the body, the data presented by Gieske and Foulkes,1974, do not necessarily have a bearing on the type of renal damage induced by chronic cadmium poisoning when no chelating agents are administered together with cadmium. But it can not be said for certain that the tubular impairment is not similar, since the mechanism for elicitation of the chronic type of lesion may involve transport of cadmium bound to metallothionein. However, it remains to be shown that the types of renal lesions produced by metallothionein-Cd and mercaptoethanol-Cd are the same and in turn correspond to those in chronic cadmium poisoning.

That a renal tubular damage can be induced by injection of metallothionein-cadmium has been shown by Nordberg, 1971, and Nordberg, Goyer and Nordberg, 1975. The last mentioned authors injected metallothionein-bound Cd in doses between 0.4 - 1.5 mg Cd/kg to mice and demonstrated prominent tubular damage by histological techniques. The glomeruli were not affected.

These authors as well as Cherian and Vostal, 1974, have reported that more cadmium is accumulated in the kidney and less in the liver after injection of metallothionein-bound cadmium compared to injection of cadmium chloride.

The prominent tubular damage is in accord with results from autoradiographic localization studies indicating that the part of the metallothionein-bound cadmium that accumulates in the kidney is localized in the renal tubules. This has been shown in recent experiments by Nordberg, Nordberg and Pannone (to be published) in which mice were injected i.v. with ^{109}Cd-labelled homologous metallothionein and studied by autoradiographical and scintillation counting techniques. During the first minutes after injection of metallothionein-bound Cd the main part the cadmium was accumulated in the kidney and subsequently excreted in the urine.

A considerable part of the cadmium hence was found in the urinary bladder in the whole body autoradiograms at 20 minutes after injection. After that period the distribution pattern was mainly unchanged. (The cadmium in the urinary bladder disappeared as the urine was voided.) Whole body measurements revealed that about half of the cadmium injected as metallothionein-bound cadmium had been excreted during the very first period after injection, while the cadmium remaining in the body (localized mainly in the kidney) had a long biological half-time.

These results are in accord with results reported by Vostal, 1975, who found that about half of ^{109}Cd-binding protein (metallothionein) injected i.v. or in the renal artery of rats was excreted in the urine.

Summary and conclusions

The protective action of metallothionein against various acute effects of cadmium has been shown with regard to such effects on various organs like testicles, liver and certain aspects of pancreatic and renal function. Such studies have been reported both on the organ and subcellular level.

Whereas metallothionein thus has a protective role against these acute manifestations of cadmium toxicity, it may be involved in the elicitation of the renal tubular impairment seen in chronic cadmium intoxication.

The above mentioned recently obtained results with regard to the distribution and toxicity of metallothionein-bound cadmium are partly confirmatory and partly contradictory to previously advanced theories concerning the mechanism for renal toxicity and excretion of cadmium in chronic cadmium poisoning. (These theories are reviewed in CITE 2nd ed.). It has been shown that metallothionein can act as a carrier for cadmium, increasing its accumulation in the kidney and giving rise to renal tubular damage. This is in accord with previous theories. However, it has also been shown that cadmium can be excreted in the urine, presumably at dose-levels of metallothionein-bound cadmium giving rise to no evident renal tubular damage. This is not in accord with previous theories. The observations supporting this last mentioned statement are not conclusive and more data are needed for a definite statement.

5.7 CARCINOGENIC AND GENETIC EFFECTS

5.7.1 Carcinogenic effects

Lucis, Lucis and Aterman, 1973, studied the tumorogenic effect of cadmium after a single dose of cadmium chloride (0.02-0.03 mM/kg) in rats. An initial testicular necrosis developed and after 48 days the first testicular tumor could be shown. Tumors were found at the site of subcutaneous injection, but not to the same extent as in the testes. The uptake of cadmium in the testes reached a maximum within about 6 hours after subcutaneous injection whereas zinc, in contrast, was accumulated continuously in testes for at least 20 days. After intrahepatic injection tumors appeared in the testes though no hepatic tumors developed.

Levy et al., 1973, presented results from a long-term study on the possible connection between cadmium exposure and cancer of the prostate. Three groups of rats, 25 in each, received a weekly subcutaneous injection of cadmium sulfate, 0.022, 0.044 and 0.087 mg Cd per rat (average weight 220 g at start and 410 g at end of exposure) for 2 years. Seventy-five animals received weekly injection of water and served as controls. Macroscopic and microscopic examinations of the prostate gland were performed. Neither in exposed animals, nor in controls were any malignant changes found in the prostate. Leydig cell tumors were found in testes in 67 % of the controls and 67-77% of the exposed groups. The authors verified in another experiment that high risk for tumors of this type is typical for the subline of the C.B. hooded rats used. In the control group 2 neoplasms were found in other organs, and in the exposed groups 3 neoplasms. In the highest exposure group other effects were seen and renal concentrations of cadmium were high. This study has added evidence for cadmium's having no carcinogenic activity, even at long-term exposure.

5.7.2 Genetic effects

It was stated in CITE, 2nd ed., that the limited literature dealing with genetic effects of cadmium was to some extent contradictory. A study on Drosophila showed no genetic effect, whereas studies from Japan on Itai-Itai patients had shown increased frequencies of chromosomal aberrations. The need for studies on people exposed to cadmium, but without complicating effects as seen in Itai-Itai disease was pointed out.

Bui, Lindsten, and Nordberg, 1975, have presented material including both cadmium exposed workers from a Swedish battery factory and Itai-Itai patients from Japan as well as control subjects in Table 5:15. No statistically significant differences in the frequency of chromosomal aberrations could be found between cadmium exposed and control subjects in either the Swedish or Japanese material.

These observations thus are at variance with previous reported observations from Japan. An explanation for this discrepancy has not yet been found.

5.8 OTHER EFFECTS

A decrease in hematocrit, hemoglobin and liver-iron was found by Freeland and Cousins, 1973, in chicks given a diet containing 75 µg Cd/g. Radioactive iron was introduced into a duodenal loop whereupon its absorption was found to be decreased. The authors concluded that at least part of the mechanism involved in cadmium anemia is a decreased absorption of iron from the intestines. Copper and zinc concentrations in liver and kidney were slightly elevated which was interpreted as showing that these metals were incorporated into the cadmium-binding protein and deposited at the same place as cadmium.

Cadmium-induced anemia in growing pigs was investigated by Pond, Walker and Kirtland, 1973. They found decreased hemoglobin levels with a diet containing 154 µgCd/g. The depression was prevented by oral or intramuscular iron-administration.

Chatterjee, Banerjee and Pal, 1973, studied the protective action of ascorbic acid on cadmium intoxication in rats. Four weeks after exposure to cadmium chloride in the diet, corresponding to a dose of 60 mg Cd/kg body weight daily, pronounced anemia, decrease in growth rate, and liver damage were observed. When the diet also contained ascorbic acid the resulting anemia was less pronounced as was the decrease in growth rate. Histological liver changes were as prominent among the cadmium-ascorbic ascorbic acid rats as among the cadmium rats.

Ogawa et al., 1973, studied the influence of cadmium chloride on the activities of carbon anhydrase, catalase, and hemoglobin level. Tap water containing 146 µg Cd/g was given ad libitum in 5 experiments to groups of 5 mice for 10, 20, 30, 60 and 90 days respectively. Control groups were given tap water without cadmium. Carbonic anhydrase activities in liver, kidney and blood were diminished significantly already after 20 days. Decrease in catalase activity was seen first after 60 and 90 days of administration of cadmium.

Diamond, Jedeikin, and Kench, 1973, found that cadmium in low concentrations stimulated, but in higher concentrations inhibited, tryptophan oxygenase.The studies were carried out in vitro.

The impact of prolonged cadmium exposure in rats (1 mg of cadmium chloride/kg body weight daily during 45 days intraperitoneally) was investigated by Singhal et al., 1974). The injections produced significant increases in the activity of liver pyruvate carboxylase, phosphopyruvate carboxylase, hexosediphosphatase, and glucose-6-phosphatase compared with control values. Withdrawal of cadmium exposure for 28 days failed to restore the enzyme activities to the control values. In treated rats, there was an increase in blood glucose and urea, contrasting with a remarkable decrease in the amount of liver glycogen, the latter being an event which persisted after the 28-day withdrawal. Excretion of protein and glucose exceeded that of controls as indicated by Combistix and Dextrostix tapes. The authors discussed the possibility that the increase in urinary protein and glucose had an enzymatic basis, at least in part.

Mills and Dalgarno, 1972, studied copper and zinc status of pregnant ewes and lambs receiving dietary concentrations of cadmium (0.7, 3.5, 6.1, and 12.3 µg/g) As in earlier experiments with rats, chicks, goats and pigs (Bunn and Matrone, 1966; Hill and Matrone, 1970; Anke et al., 1971), the cadmium treated ewes showed a decreased copper concentration in liver. This decrease was pronounced at the high dose level but could be shown also after medium exposure. As could be expected, lamb liver at birth did not show any increase in cadmium concentration. Copper concentration in liver was reduced in lambs whose mothers had received 7.1 or 12.3 µg Cd/g in the diet. What complicates the interpretation of the studies is that the diet (herbage) contained high amounts not only of cadmium but also of zinc. The zinc concentration was considerably more than the threshold concentrations (200-400 µg/g) above which it induces clinical effects attributable to copper deficiency in rats and chicks (Bunn and Matrone, 1966; Hill and Matrone, 1970).

TABLE 5:1

MAIN PATHOLOGICAL FINDINGS AFTER EXPERIMENTAL CHRONIC CADMIUM POISONING IN RATS (From Kawai and Fukuda, 1974).

Cd conc in drinking water μg/g	Expo-sure time months	KIDNEY			BONE				LIVER		LIVER + KIDNEY
		pathol. finding	Cd μg/g	Cd amount μg	Atrophy	Corti-cal thick. %	Ca mg/g	Cd μg/g	Cd μg/g	Cd amt. μg	Cd total amt. μg
10	8.5	·–	12.3	41	±	27.6	129	1.07	4.2	75	116
50	"	–·+	38.4	142	+	26.3	128	1.44	29.8	500	692
100	"	+	100.1	303	+	23.7	125	2.39	73.1	987	1290
200	8.5	+ E.A.	156.3	478	+	22.0	113	3.48	143.1	2064	2532
200	18	++ F.D.	136.8	467	++	19.8	114	2.87	234.7★ (A)	2283	2750

A: Atrophy D: Changes E: Edema F: Fibrosis rat SD-JCL† ★29.62/4205

TABLE 5:2

URINARY IMINO ACID EXCRETION IN DIFFERENT PERSONS (From Sano and Iguchi, 1974).

		Itai-itai Patients, Fuchu area							Cadmium-exposed persons, Ikuno area							Cadmium Workers					Controls	
		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	1	2
Hyp mg/1	Total	91	52	117	67	61	212	48	48	10	246	12	84	62	91	49	23	6	22	20	1	0
	Free	51	37	61	38	38	112	40	23	trace	153	trace	58	38	35	trace	11	trace	trace	3	trace	
	Bound	40	15	56	29	23	100	8.0	25	10	93	12	26	24	56	49	12	6	22	17	1	0
Pro. mg/1	Total	389	153	927	551	/	933	234	166	57	527	36	207	129	527	107	74	16	40	53	2	0
	Free	144	115	571	251	483	590	129	72	28	175	trace	153	56	175	trace	23	trace	trace	15	trace	
	Bound	245	38	356	300	/	343	95	94	29	342	36	54	73	342	107	51	16	40	38	2	0

TABLE 5:3

PRINCIPAL CHARACTERISTICS OF MALE WORKERS (E3) EXPOSED > 20 YEARS TO Cd AND OF CORRESPONDING MALE CONTROL WORKERS (C3)★ (From Lauwerys et al., 1974).

	Cd-Exposed (E3) (Smokers)	Controls (C3) (Smokers)
No. of workers	22	22
Age, yr		
Mean	51.5 ± 1.42	51.4 ± 1.56
Median	51.0	51.0
Height, cm	168.6 ± 0.99	170.5 ± 0.85
Weight, kg	74.4 ± 1.53	76.0 ± 2.27
No. of cigarettes smoked per day	13.0 ± 1.35	16.6 ± 1.48
Duration of smoking, yr	28.5 ± 1.82	25.9 ± 1.23
Duration of factory employment, yr	27.8 ± 1.58	29.1 ± 2.17

★ Values expressed as mean ± SE.

TABLE 5:4

PULMONARY FUNCTION TESTS IN Cd-EXPOSED (E3) AND CONTROL (C3) WORKERS● (From Lauwerys et al., 1974).

	Cd-Exposed (E3) (Smokers)	Controls (C3) (Smokers)	p†
No. of workers	22	22	
FVC, liters	3.61 ± 0.15	4.01 ± 0.11	< .05
$FEV_{1.0}$, liters	2.51 ± 0.12	2.85 ± 0.09	< .05
$T_{0.50}$, sec	0.40 ± 0.08	0.37 ± 0.08	> .05
PEFR, liters/sec	7.32 ± 0.37	8.13 ± 0.26	< .05
V_{50}, liters/sec	3.12 ± 0.28	3.64 ± 0.27	> .05
V_{25}, liters/sec	0.96 ± 0.10	1.07 ± 0.10	> .05

● Values expressed as mean ± SE.

† Values obtained from Student t-test.

TABLE 5:5

BIOLOGICAL INDICES IN Cd-EXPOSED (E3) AND CONTROL (C3) WORKERS* (From Lauwerys et al., 1974).

	Cd-Exposed (E3) (Smokers)	Controls (C3) (Smokers)	P†
No. of workers	22	22	
Cd concentration in urine, µg/g creatinine	31.0 ± 4.30	2.2 ± 0.20	< .05
Cd concentration in blood, ng/g	27 ± 3.0	7 ± 1.0	< .05
γ -galactosidase level in plasma, units/hr/ml	97.0 ± 5.00	66.0 ± 5.00	< .05
LDH level in plasma, units/min/ml	71.0 ± 4.50	58.5 ± 2.50	< .05
Catalase level in plasma, units/hr/ml	2.4 ± 0.03	2.4 ± 0.02	> .05
CHE level in plasma, µmols thiocholine/min/ml	2.1 ± 0.13	2.2 ± 0.10	> .05
RBC-ACHE, µmols thiocholine/min/ml	7.4 ± 0.22	8.2 ± 0.27	< .05
Hematocrit, %	41.2 ± 0.70	44.8 ± 0.70	< .05
No. of workers with abnormal urinary protein electrophoresis	15	0	< .05

* Values expressed as mean ± SE.

† Values obtained from Student t-test when $\sigma 1 \neq \sigma 2$.

TABLE 5:6

PROTEINURIA AND Cd CONCENTRATION IN BLOOD AND URINE OF MALE WORKERS● (From Lauwerys et al., 1974).

	Controls	P+	Exposed Normal Electro-phoresis	P+	Abnormal Electro-phoresis
No. of workers	50		30		19
Proteins in urine, mg/g creatinine	153 ± 8	>.05	158 ± 8	<.05	441 ± 79
Cd concentration in urine, μg/g creatinine	1.72 ± 0.15	<.05	11.70 ± 1.60	<.05	48 ± 10
Cd concentration in blood, ng/g	7 ± 0.7	<.05	24.7 ± 4.9	<.05	33.1 ± 4.9

● Values expressed as mean ± SE

+ Values obtained from Student t-test when $\sigma 1 \neq \sigma 2$. Each value is inserted between two values that are compared.

TABLE 5:7

INDIVIDUAL RESULTS OF URINE ANALYSIS OF 19 WORKERS IN A CADMIUM PIGMENT PLANT (Compiled from two studies by Harada 1973, and Harada et al., 1974).

Exposure time years	TCA-result	Increase in globulin fraction of electrophoresis
0.75	-	-
0.75	-	-
1.25	±	-
1.75	-	±
2.0	-	+
2.25	-	-
2.25	-	-
3.2	-	-
3.2	-	-
3.4	±	-
3.4	±	±
5.0	-	+
5.9	±	+
6.5	-	±
10.5	+	+
11.75	+	+
13.75	-	+
15.5	-	+
22.0	+	+

TABLE 5:8

URINARY EXCRETION OF TOTAL PROTEINS, CADMIUM AND β_2-MICROGLOBULIN IN ITAI-ITAI PATIENTS, OBSERVATION PATIENTS AND CONTROLS-JAPANESE FARMERS IN ANOTHER AREA (From Shiroishi et al., to be published).

	Itai-itai Patients	Observation Patients	Controls
Number of persons	10	32	17
Total protein (Kingsbury-Clark) mg/g creatinine	1066 ± 402	943 ± 587	64 ± 148
Cadmium ug/g creatinine	----	22.4 ± 12.8	8.2±11.9
β_2-microglobulin mg/g creatinine	98.9 ± 33.4	51.1 ± 36.3	0.32±0.5

TABLE [illegible]

AUTOPSY CASES DIAGNOSED CLINICALLY AS ITAI-ITAI DISEASE (from Kajikawa et al., 1974).

Name	No. of Autopsy	Age	Sex	Time of Autopsy	Bone	Kidney	Parathyroid	Other Organs	Cause of death	Duration (Yrs)	Address	Urine
1.T.M.	3412	62	F	1957	OM +++ OP ++	Pyelonephritis Arteriosclerosis Metastatic calcification	Normal	Pulmonary congestion and edema	Emaciation	12	Fuchu-machi	P - P -
2.T.Y.	3479	62	F	1959	OM +++ OP ++	Pyelonephritis Arteriosclerosis Metastatic calcification	Slightly hyper-plastic	Pulmonary congestion and edema Enterocolitis	Emaciation	5	Fuchu-machi	P - S -
3.T.K. ••	3616	49	F	1958	Osteitis fibrosa	Nephrocalcinosis Pyelonephritis Hydronephrosis	Hyperplasia (adenoma)	Metastatic calcification	Uremia	10	Izumi-machi, Toyama City	P +˜- S -
4.S.M. ••	4257	44	[illegible]	1963	Osteitis fibrosa with pseudofracture	Nephrocalcinosis	Hyperplasia (secondary?) adenomatous	Chronic pancreatitis, Gastritis and colitis, Thrombosis of the portal vein	Portal thrombosis(?)	5	Nyuzen-machi	P + S -
5.I.F.	4574	73	F	1965	OM + OP + OS +	TN +++	Normal	Chronic pancreatitis Chronic hepatitis	Uremia	12	Yoshikura (Shimbo), Toyama City	P + S -
6.S.M.	5447	61	F	1965	OM + OP + (Pseudofracture in X-ray film)	Suppurative pyelonephritis	Normal	Verrucous endocarditis (Mitral and aortic stenosis with insufficiency)	Cardiac failure	11	Yoshikura (Shimbo), Toyama City	P + S -
7.S.S.	5313	79	F	1966	OM + OP + OS +	TN +	Normal	Cancer of the stomach	Cancer	8	Tonan-kami-kuriyama, Toyama City	P + S ++
8.T.T.	——	73	F	1968	OM ++ OP ± (ribs)	TN ++	Normal	Cancer of the stomach	Cancer	8	Fuchu-machi	P ++ S ++
9.H.S.	5677	75	F	1969	OM ± OP + OS +	TN ++	Normal	Bronchopneumonia Fibrinous pleuritis (1) Ulcerative colitis	Uremia	2 (?)	Fuchu-machi	P ++ S ++
10.I.T.	6128	68	F	1971	OM ++ OP ++ OS ±	TN +++	Normal	Anemia Hypertrophy of the heart Pulmonary edema	Uremia	16	Shimbo, Toyama City	P ++ S ++
11.I.S. ••	6555	72	F	1972	OP ++	Arteriosclerosis	Normal	Anemia Malnutrition	Suicide by hanging	21	Fuchu-machi	P + S ++

OM: Osteomalacia
OP: Osteoporosis
OS: Osteosclerosis (including healed pseudofracture)
TN: Tubular nephropathy (tubular atrophy and dilatation with proteinaceous casts, and interstitial fibrosis)
P : Proteinuria
S : Glucosuria
•• : Possibly different disorders from Itai-Itai-disease

TABLE 5:10

DOSE RESPONSE RELATIONSHIP BETWEEN DECREASE IN P-450 ACTIVITY AND Cd DOSE (From Unger and Clausen, 1973).

Table 1

i.p. administration of $Cd(NO_3)_2,4HOH$ in saline solution	No. of animals	Specific P-450 O-demethylation activity (nmoles/mg liver microsome protein/min)		Statistical evaluation of mean values compared with data of untreated animals		No. of deaths within 72 hours
		Mean	Standard deviation	Variance-ratio P(F)	Student's P(t)	
nil	4	9.7	1.2	-	-	-
50 mg/kg body weight	4	-	-	-	-	all dead within 8 hours
10 mg/kg b.w.	5	2.6	1.2	<0.05	<0.0005	none
2.8 mg/kg b.w.	2	2.0	0.5	<0.05	<0.005	none
1 mg/kg b.w.	8	5.3	1.8	<0.05	<0.025	none
100 μg/kg b.w.	3	7.9	1.7	<0.05	<0.20	none
10 μg/kg b.w.	8	7.6	1.3	<0.05	<0.05	none
100 ng/kg b.w.	9	7.9	3.4	<0.05	<0.30	none
10 ng/kg b.w.	4	8.2	1.0	<0.05	<0.10	none
1 ng/kg b.w.	10	8.5	3.0	<0.05	<0.40	none
0.1 ng/kg b.w.	4	9.6	3.2	<0.05	<0.40	none

TABLE 5:11

EFFECT OF CADMIUM CONCENTRATION ON 1,25-DIHYDROXYCHOLECALCIFEROL SYNTHESIS BY CHICK KIDNEY MITOCHONDRIA (From Feldman and Cousins, 1973).

Cadmium μMolar[a]	% 1,25-DHCC
0	65.0
25	38.0
50	17.2
55	14.5
60	13.3
65	11.1
70	10.4
75	9.7
100	6.0
125	2.3

[a] Cadmium solutions were incubated with the mitochondria for 3 min prior to addition of [^{3}H]-25-HCC.

TABLE 5:12

PRODUCTION OF 1,25-DHCC IN KIDNEYS OF MICE FED DIFFERENT AMOUNTS OF CADMIUM (From Kimura et al., in press).

Cd in diet	Feeding time	Percent radioactivity		
		25-OH-D_3	$24,25\text{-(OH)}_2\text{-D}_3$	$1,25\text{-(OH)}_2\text{-D}_3$
ppm	weeks	%	%	%
0	3	68.6	1.7	30.3
10	3	64.4	1.2	34.4
30	3	66.0	1.9	30.5
30	8	69.7	2.0	24.5
300	3	82.1	1.6	16.4

TABLE 5:13

SUMMARY OF BREEDING EXPERIMENTS IN MICE EXPOSED TO TRACE ELEMENTS (From Schroeder and Mitchener, 1971).

	Control	Cadmium
F_1 generation		
No. of litters	19	14
Pair age at first litter, days	64	80.0
Interval between litters, days	30	34.6
Average litter size	11.0	13.1
M-F ratio	0.94	1.05
F_2 generation		
No. of litters	23	11
Pair age at first litter, days	64	69
Interval between litters, days	28	56
Average litter size	10.3	9.2
M-F ratio	1.03	0.78
F_3 generation		Discontinued
No. of litters	22	
Pair age at first litters, days	62	
Interval between litters, days	28	
Average litter size	10.5	
M-F ratio	1.00	

TABLE 5:14

DEATHS AND ABNORMALITIES OF MICE BRED WHILE EXPOSED TO TRACE ELEMENTS (From Schroeder and Mitchener, 1971).

	Control	Cadmium
F_1 generation		
Maternal deaths	0	0
Dead litters	0	0
Young deaths	0	39+
Failures to breed	0	0
Runts	0	25+
No. of mice	209	184
F_2 generation		
Maternal deaths	1	2
Dead litters	0	2
Young deaths	6	48+
Failures to breed	0	3
Runts	2	9*
No. of mice	248	101
F_3 generation		Discontinued
Maternal deaths	0	
Dead litters	0	
Young deaths	1	
Failures to breed	0	
Runts	0	
No. of mice	230	
Total No. of mice	687	285

*Differs from controls by χ^2 analysis; P < 0.0005

+P <0.0001

TABLE 5:15

RESULTS OF CADMIUM ANALYSIS OF BLOOD AND CYTOGENETIC ANALYSIS OF LYMPHOCYTES FROM CADMIUM EXPOSED AND CONTROL SUBJECTS (From data by Bui, Lindsten and Nordberg, 1975).

Case No	Cadmium in whole blood ng/g	Total No of cells analyzed	No of cells with structural aberrations	Mean frequency %
I 1	15.5	97	11	
I 2	17.0	99	6	
I 3	16.6	100	4	
I 4	28.8	100	5	6.6
C 1	5.3	100	7	
C 2	6.1	82	5	
C 3	4.4	100	7	
C 4	4.4	98	4	6.0
E 1	24.7	100	1	
E 2	46.0	100	2	
E 3	61.0	100	2	
E 4	30.0	100	2	
E 5	26.7	100	3	2.0
K 1	2.2	100	5	
K 2	3.2	100	0	
K 3	1.4	100	9	4.7

I 1-4 = Itai-Itai disease cases from Toyama prefecture Japan.

C 1-4 = Control subjects from Toyama prefecture Japan.

E 1-5 = Cadmium exposed workers from a Swedish battery factory.

K 1-3 = Control workers from a Swedish battery factory.

TABLE 5:16

INCIDENCE OF MALFORMATIONS AMONG FETUSES DUE TO $CdCl_2$ INJECTION. (From Ishizu et al., 1973).

Dose Cd injected	Malformed	Dead
5 mg/kg	59.0 %	17.5 %
2.5 mg/kg	19.5 %	12.0 %
0.6 mg/kg	0.97%	11.9 %
0.3 mg/kg	0	9.9 %
	0	9.7 %

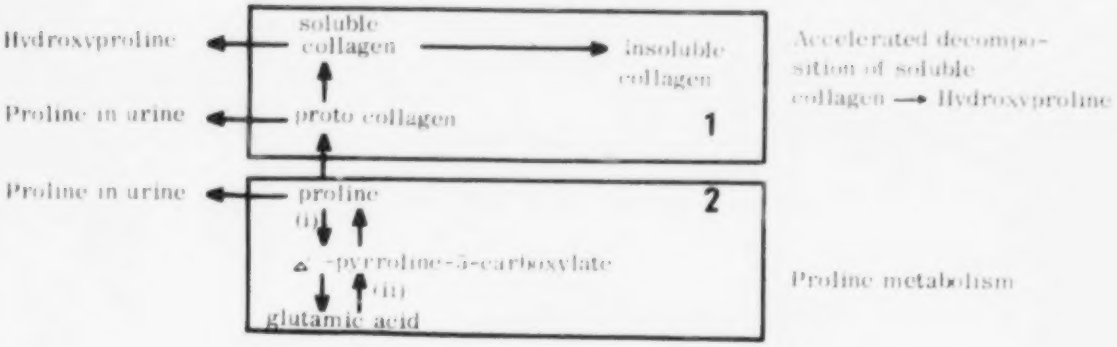
Hydroxyproline
soluble collagen
insoluble collagen
Accelerated decomposition of soluble collagen → Hydroxyproline
Proline in urine
proto collagen
1
Proline in urine
proline
2
(i)
Δ -pyrroline-5-carboxylate
(ii)
glutamic acid
Proline metabolism

FIGURE 5:1 Metabolic pathways of imino acids and collagen. (From Sano and Iguchi, 1974).

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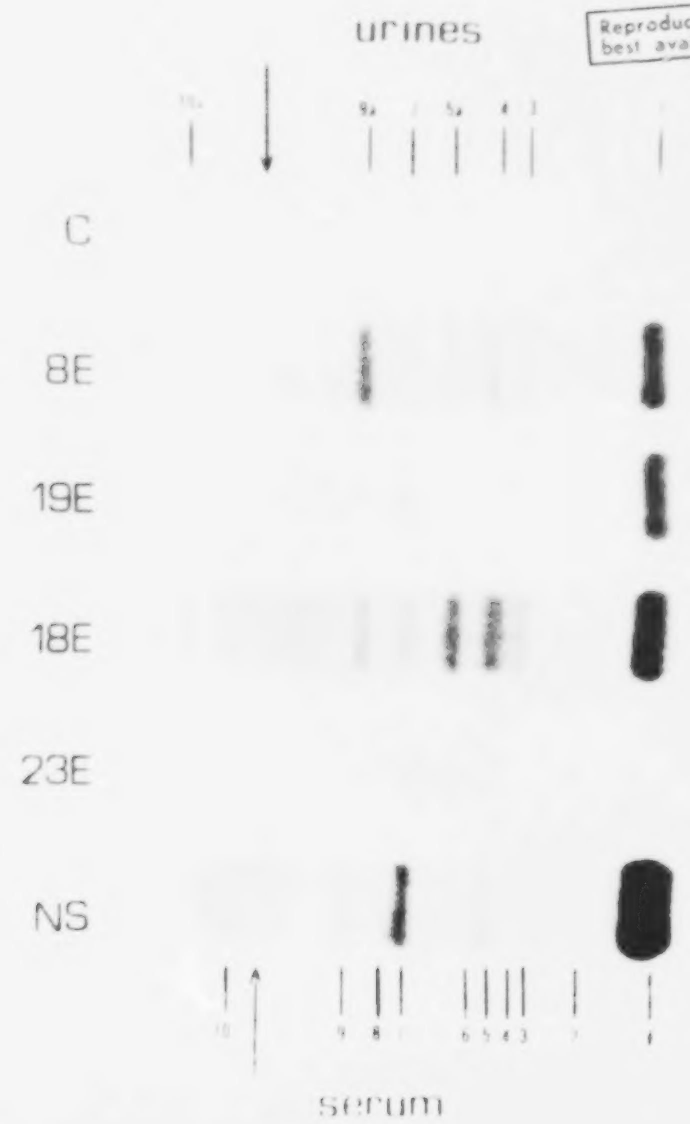


FIGURE 5:2 Electrophoresis on agarose gel of urinary proteins of four Cd-exposed workers of E3 group: 18E (1,254 mg/g creatinine), 8E (851 mg/g creatinine), 19E (480 mg/g creatinine), and 23E (337 mg/g creatinine); and of urinary (C) (134 mg/g creatinine) and serum (NS) proteins of control worker. Serum proteins are (1) albumin, (2) α_1-antitrypsin, (3) α_2-zinc-glycoprotein, (4) α_2-HS-glycoprotein, (5) α_2-macroglobulin, (6) haptoglobin, (7) transferrin, (8) β -lipoprotein, (9) β-1_c-globulin, and (10) γ-globulin. Slight amount of urinary proteins in control, mainly albumin, not visible. Cadmium-exposed workers 8E and 18E showed 1, 3, 4, and 7 among their urinary proteins, as well as low molecular weight proteins α_2-microglobulin (5a) and β_2-microglobulin (9a). Only 18E had post-γ-globulin (10a). Electrophoretic pattern of 23E resembled that of 19E, but urinary protein concentration was lower; 19E showed only 1, 3, 4, and 7. (From Lauwerys et al., 1974).

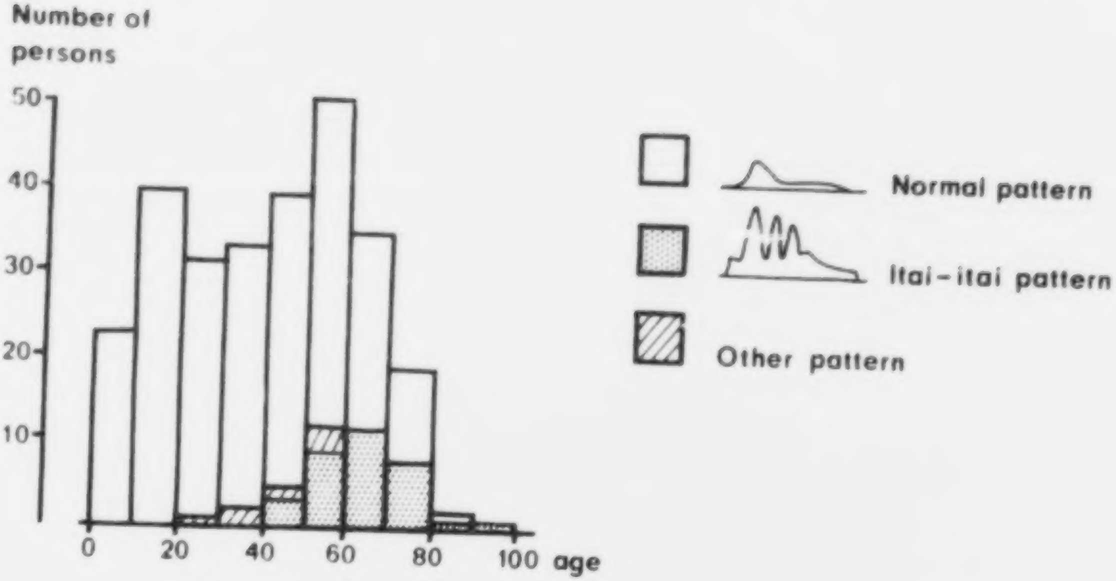


FIGURE 5:3 The distribution of urinary protein patterns on disc electrophoresis in relation to age among inhabitants of the Itai-itai disease endemic area. (From Shiroishi et al., 1972).

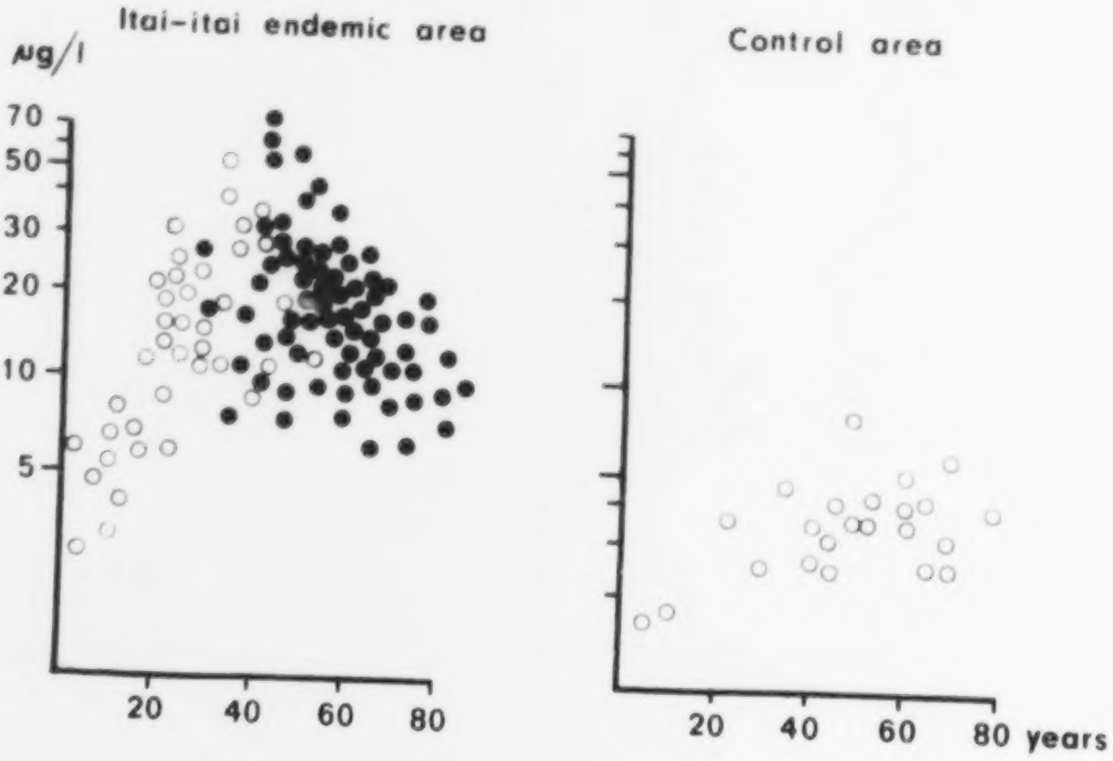
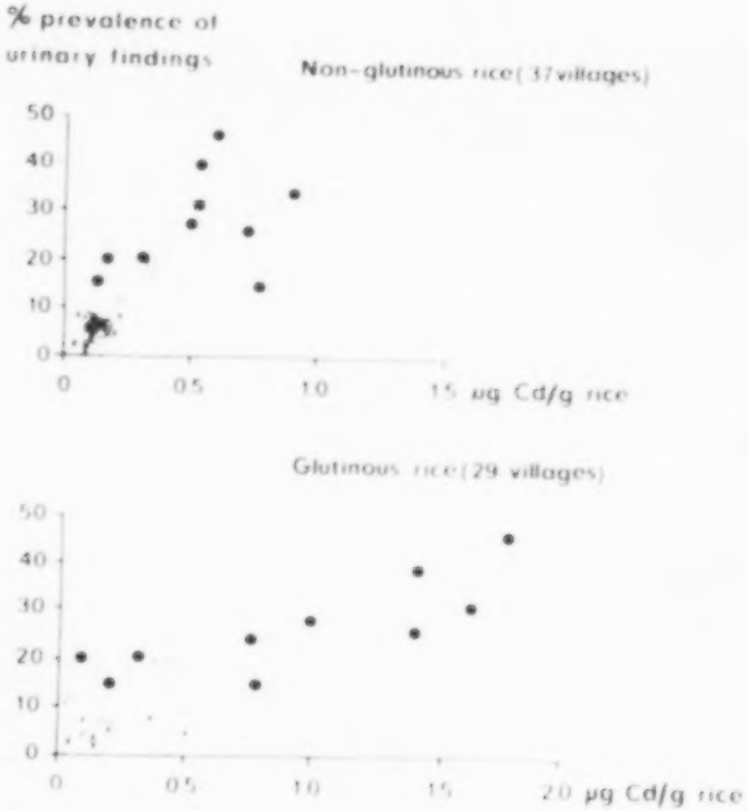


FIGURE 5:4 Urinary cadmium excretion in relation to residence time in area. Filled circles = Itai-itai patterns on disc electrophoresis. (From Fukuyama et al., 1972).



● Villages with high prevalence of Itai-itai disease.
Villages with low prevalence of Itai-itai disease.
△ Villages with no Itai-itai disease within the polluted area.
X Villages outside polluted area.

FIGURE 5:5 Prevalence of concurrent proteinuria and glucosuria among persons over 50 years of age within villages in relation to cadmium concentration in rice in villages with varying prevalences of Itai-itai disease. (Redrawn from Fukushima et al., 1973).

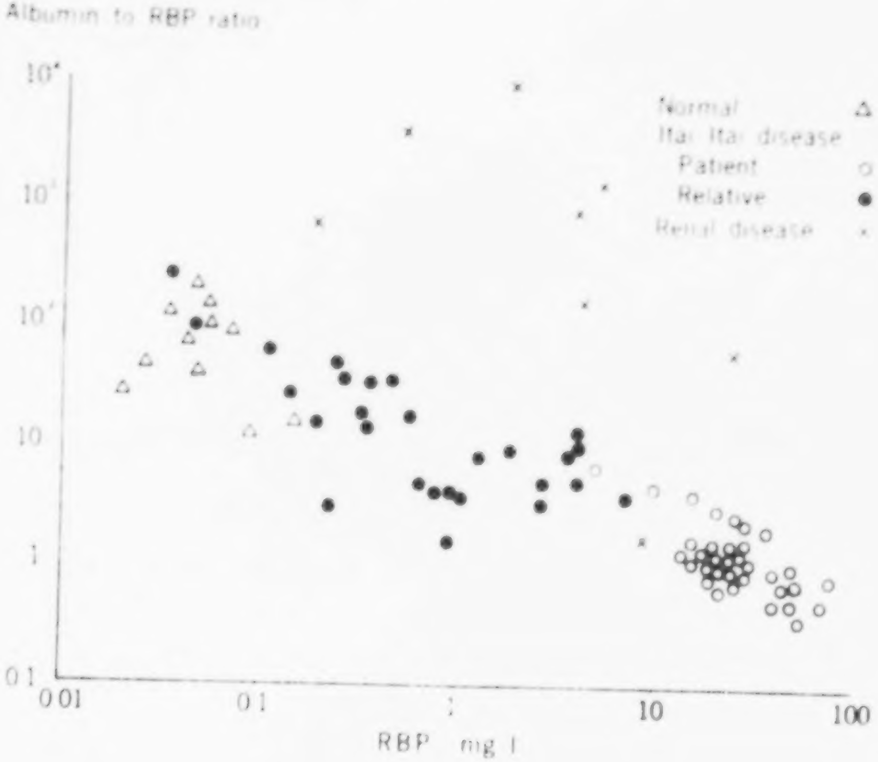


FIGURE 5:6 Urinary RBP concentration and the ratio albumin/RBP in normal and some proteinuric conditions. (From Kanai et al., 1972).

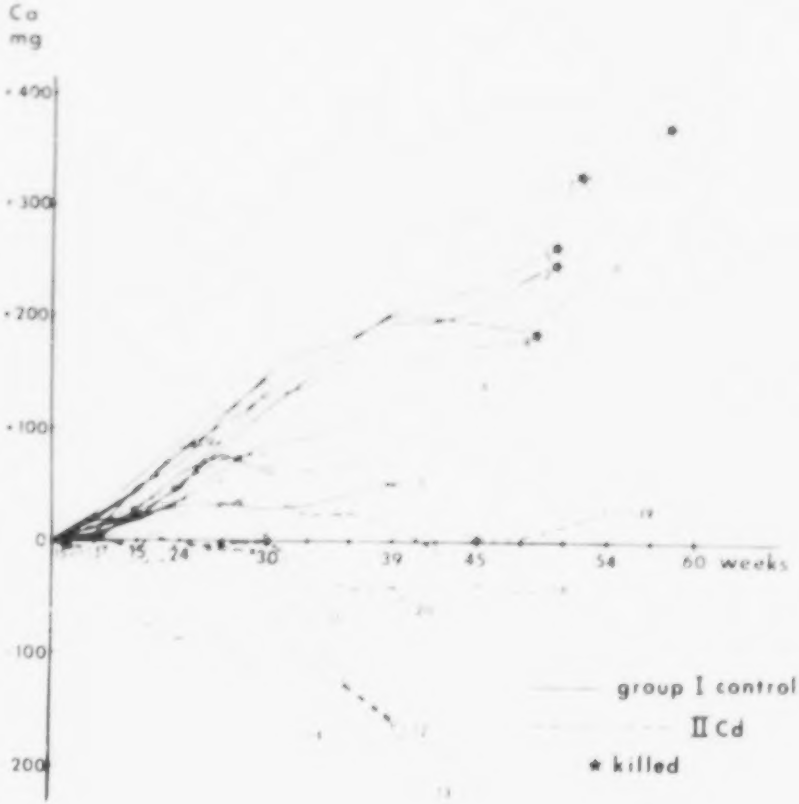


FIGURE 5:7 Balance of calcium metabolism of rats given two kinds of diets (calcium deficient with and without added cadmium). Data are given for individual rats. (From Kobayashi, 1973).

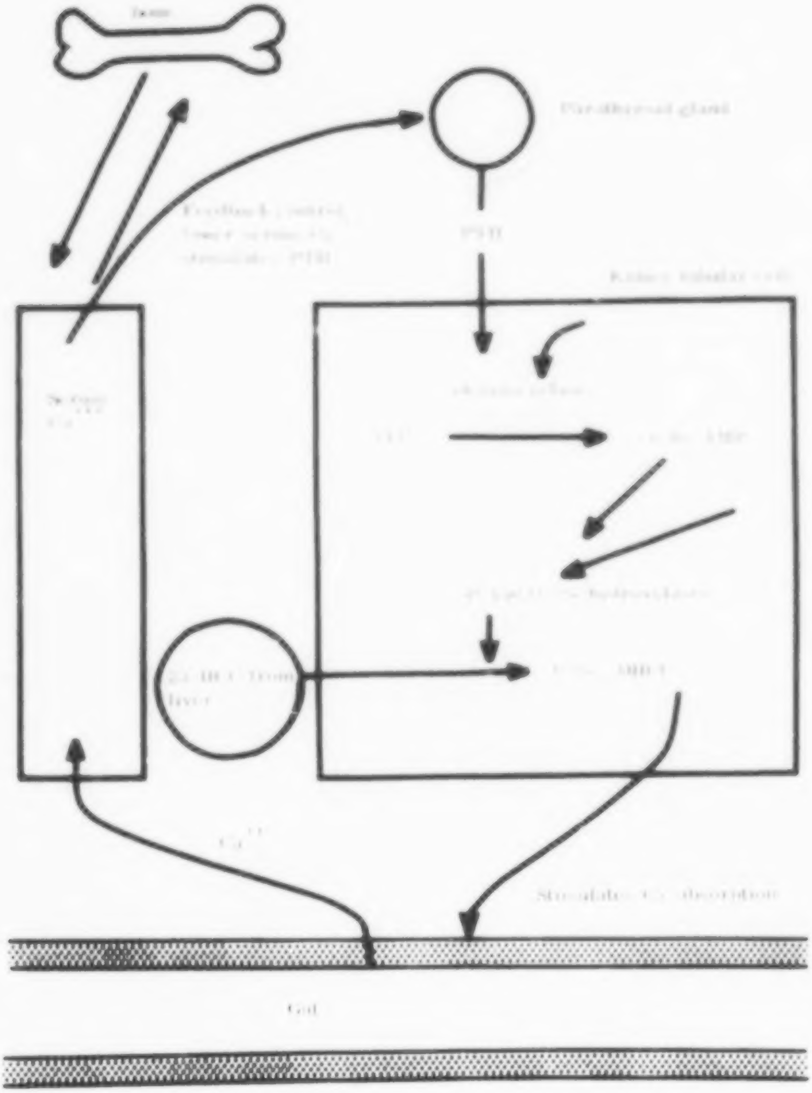

Bone
Parathyroid gland
Feedback control,
lower serum Ca
stimulates PTH
PTH
Kidney tubular cell
Serum
Ca++
25-HCC from
liver
Ca++
Stimulates Ca absorption
Gut

FIGURE 5:8 A schematic drawing of the pathways involved in Vitamin D metabolism.

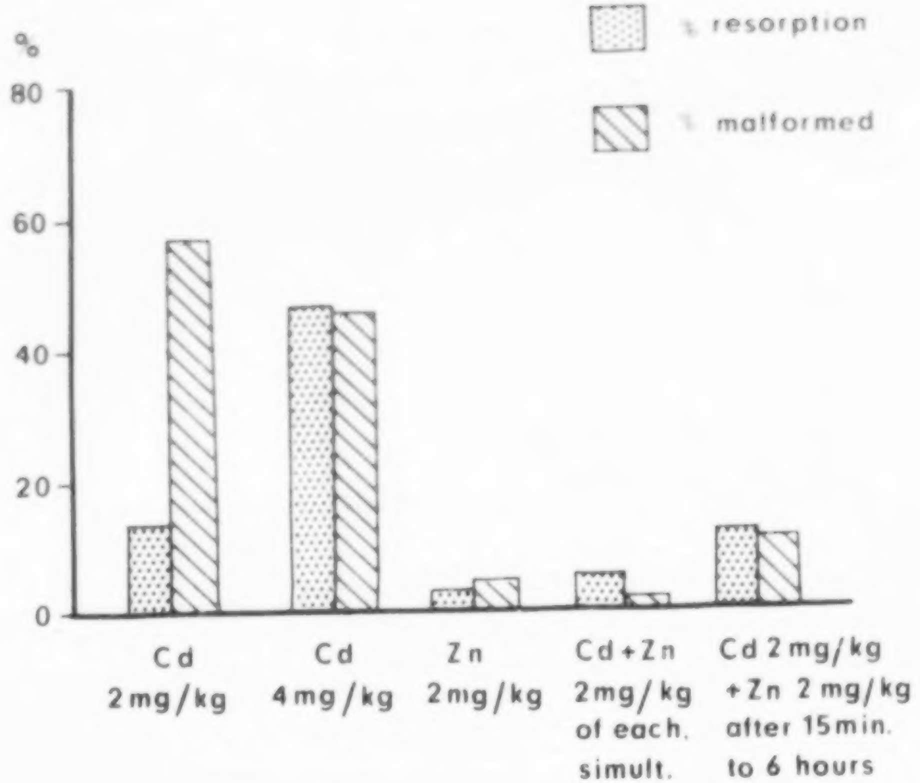


FIGURE 5:9 A comparison of the teratogenic and embryocidal effects of cadmium, zinc, and cadmium-zinc on embryonic development in the golden hamster. The protection afforded by zinc against the teratogenic activity of cadmium diminishes as the interval between injections increases. (From Ferm and Carpenter, 1968).

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